APPENDICES

Table 1 Analysis of variance table by two factorial treatment effects and interaction of the growth of nematophagous fungi on six media at 3 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.036	0.0180	2.74	0.0698
Media	5	19.142	3.3884	582.92	0.0000
Isolate	7	82.578	11.7968	1796.19	0.0000
Media*Isolate	35	23.180	0.6623	100.84	0.0000
Error	94	0.617	0.0066		
Total	143	540.387			
Coefficient of variance			2.88		

Table 2 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi on six media at 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.048	0.0238	3.33	0.0401
Media	5	76.570	15.3139	2140.97	0.0000
Isolate	7	387.557	55.3653	7740.39	0.0000
Media*Isolate	35	75.540	2.1583	301.74	0.0000
Error	94	0.672	0.0072		
Total	143	540.387			
Coefficient of variance			1.53		

Table 3 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi on six media at 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.039	0.0194	3.73	0.0275
Media	5	52.086	10.4172	2006.93	0.0000
Isolate	7	591.240	84.4629	16272.3	0.0000
Media*Isolate	35	79.047	2.2585	435.11	0.0000
Error	94	0.488	0.0052		
Total	143	722.900			
Coefficient of variance			1.01		

Table 4 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi on six media at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.003	0.0015	0.52	0.5964
Media	5	5.659	1.1318	403.37	0.0000
Isolate	7	405.047	57.8639	20622.6	0.0000
Media*Isolate	35	53.522	1.5292	545.00	0.0000
Error	94	0.264	0.0028		
Total	143	464.494			
Coefficient of variance			0.68		

Table 5 Analysis of variance table by factorial treatment effects and interaction of the sporulation of nematophagous fungi on six media at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0263	0.01316	17.75	0.0000
Media	5	1.2645	0.25290	341.12	0.0000
Isolate	7	33.0182	4.71688	6362.18	0.0000
Media*Isolate	35	9.9286	0.28368	382.62	0.0000
Error	94	0.0697	0.00074		
Total	143	44.3073			
Coefficient of variance			1.89		

Table 6 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at five temperatures at 3 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0033	0.00165	0.16	0.8491
Temperature	4	22.4310	5.60776	558.36	0.0000
Isolate	7	22.1040	3.15771	314.41	0.0000
Temperature*Isolate	28	16.6603	0.59501	59.24	0.0000
Error	78	0.7834	0.01004		
Total	119	61.9820			
Coefficient of variance			5.90		

Table 7 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at five temperatures at 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.070	0.0349	1.60	0.2080
Temperature	4	154.265	38.5662	1771.03	0.0000
Isolate	7	123.270	17.6100	808.68	0.0000
Temperature*Isolate	28	76.965	2.7488	126.23	0.0000
Error	78	1.699	0.0218		
Total	119	356.269			
Coefficient of variance			4.07		

Table 8 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at five temperatures at 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.059	0.0297	1.31	0.2746
Temperature	4	348.125	87.0312	3852.05	0.0000
Isolate	7	226.508	32.3583	1432.20	0.0000
Temperature*Isolate	28	142.054	5.0734	224.55	0.0000
Error	78	1.762	0.0226		
Total	119	718.508			
Coefficient of variance			3.01		

Table 9 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at five temperatures at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.022	0.011	0.50	0.6068
Temperature	4	456.483	114.121	5176.75	0.0000
Isolate	7	323.795	46.256	2098.28	0.0000
Temperature*Isolate	28	206.601	7.379	334.71	0.0000
Error	78	1.719	0.022		
Total	119	988.620			
Coefficient of variance			2.34		

Table 10 Analysis of variance table by factorial treatment effects and interaction of the sporulation of nematophagous fungi at five temperatures at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0087	0.00434	16.46	0.0000
Temperature	4	0.8491	0.21228	805.85	0.0000
Isolate	7	66.9757	9.56795	36321.0	0.0000
Temperature*Isolate	28	10.0537	0.35906	1363.03	0.0000
Error	78	0.0205	0.00026		
Total	119	77.9077			
Coefficient of variance			1.14		

Table 11 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at four different light regimes 3 days after incubation

Source	df	SS	MS	F	P
Rep	2	0.023	0.0114	1.63	0.2033
Light	3	6.262	2.0874	300.53	0.0000
Isolate	7	110.016	15.7166	2262.83	0.0000
Light*Isolate	21	11.638	0.5542	79.79	0.0000
Error	62	0.431	0.0069		
Total	95	128.370			
Coefficient of variance			2.80		

Table 12 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at four different light regimes 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.114	0.0570	7.80	0.0009
Light	3	26.977	8.9925	1231.55	0.0000
Isolate	7	375.630	53.6614	7349.12	0.0000
Light*Isolate	21	54.304	2.5859	354.15	0.0000
Error	62	0.453	0.0073		
Total	95	457.478			
Coefficient of variance			1.55		

Table 13 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at four different light regimes 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.048	0.0239	6.37	0.0031
Light	3	23.294	7.7647	2072.45	0.0000
Isolate	7	452.563	64.6519	17256.0	0.0000
Light*Isolate	21	79.701	3.7953	1012.98	0.0000
Error	62	0.232	0.0037		
Total	95	555.838			
Coefficient of variance			0.88		

Table 14 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at four different light regimes 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.001	0.0004	0.13	0.8747
Light	3	12.973	4.3243	1392.75	0.0000
Isolate	7	327.909	46.8442	15087.5	0.0000
Light*Isolate	21	60.360	2.8743	925.74	0.0000
Error	62	0.193	0.0031		
Total	95	401.435			
Coefficient of variance			0.75		

Table 15 Analysis of variance table by factorial treatment effects and interaction of the sporulation of nematophagous fungiat four different light regimes 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0224	0.01121	8.46	0.0006
Light	3	0.1884	0.06278	47.40	0.0000
Isolate	7	22.0193	3.14562	2374.72	0.0000
Light*Isolate	21	1.3405	0.06383	48.19	0.0000
Error	62	0.0821	0.00132		
Total	95	23.6527			
Coefficient of variance			2.83		

Table 16 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at eight pH levels 3 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.119	0.0593	11.00	0.0000
pH	7	59.587	8.5124	1578.09	0.0000
Isolate	7	215.026	30.7179	5694.69	0.0000
pH*Isolate	49	17.144	0.3499	64.86	0.0000
Error	126	0.680	0.0054		
Total	191	292.555			
Coefficient of variance			2.18		

Table 17 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at eight pH levels 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.047	0.0236	2.97	0.0549
pH	7	144.060	20.5800	2594.23	0.0000
Isolate	7	580.161	82.8802	10447.5	0.0000
pH*Isolate	49	25.875	0.5281	66.57	0.0000
Error	126	1.000	0.0079		
Total	191	751.143			
Coefficient of variance			1.60		

Table 18 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi at eight pH levels 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.034	0.017	1.57	0.2118
pH	7	147.436	21.062	1968.66	0.0000
Isolate	7	731.571	104.510	9768.41	0.0000
pH*Isolate	49	30.874	0.630	58.89	0.0000
Error	126	1.348	0.011		
Total	191	911.262			
Coefficient of variance			1.45		

Table 19 Analysis of variance table by factorial treatment effects and interaction of the growth of nematophagous fungi growth at eight pH levels 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	1.823E-04	0.0001	0.03	0.9746
pH	7	72.8029	10.4004	2935.04	0.0000
Isolate	7	504.876	72.1251	20354.0	0.0000
pH*Isolate	49	39.0248	0.7964	224.76	0.0000
Error	126	0.44648	0.0035		
Total	191	617.150			
Coefficient of variance			0.76		

Table 20 Analysis of variance table by factorial treatment effects and interaction of the sporulation of nematophagous fungi at eight pH levels 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0230	0.01148	12.63	0.0000
pH	7	0.6061	0.08659	95.28	0.0000
Isolate	7	62.1918	8.88454	9776.23	0.0000
pH*Isolate	49	6.9312	0.14145	155.65	0.0000
Error	126	0.1145	0.00091		
Total	191	69.8666			
Coefficient of variance			2.37		

Table 21 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi by six insecticides at 3 days after incubation

Source	df	SS	MS	F	P
Replication	2	8	4.1	1.46	0.2345
Isolate	7	363456	51922.3	18272.4	0.0000
Insecticide	5	5419	1083.7	381.38	0.0000
Rate	3	483	160.8	56.60	0.0000
Isolate*Insecticide*Rate	176	48369	274.8	96.72	0.0000
Error	382	1085	2.8		
Total	575	418820			
Coefficient of variance			17.75		

Table 22 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi by six insecticides at 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	20	10.1	3.61	0.0281
Isolate	7	52488	7498.2	2683.48	0.0000
Insecticide	5	345159	69031.8	24705.2	0.0000
Rate	3	18349	6116.3	2188.91	0.0000
Isolate*Insecticide*Rate	176	117987	670.4	239.92	0.0000
Error	382	1067	2.8		
Total	575	535070			
Coefficient of variance			2.62		

Table 23 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi by six insecticides at 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	14	7.2	2.89	0.0569
Isolate	7	47709	6815.6	2736.65	0.0000
Insecticide	5	355402	71080.4	28540.5	0.0000
Rate	3	16878	5625.9	2258.95	0.0000
Isolate*Insecticide*Rate	176	112834	641.1	257.42	0.0000
Error	382	951	2.5		
Total	575	533789			
Coefficient of variance			2.46		

Table 24 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi by six insecticides at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	13	6.4	4.09	0.0175
Isolate	7	53669	7667.0	4860.11	0.0000
Insecticide	5	375618	75123.5	47620.6	0.0000
Rate	3	20470	6823.4	4325.36	0.0000
Isolate*Insecticide*Rate	176	189334	1075.8	681.92	0.0000
Error	382	603	1.6		
Total	575	639707			
Coefficient of variance			2.12		

Table 25 Analysis of variance table by factorial treatment effects and interaction of the sporulation of nematophagous fungi by six insecticides at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.006	0.0028	9.47	0.0001
Isolate	7	85.279	12.1827	40591.9	0.0000
Insecticide	5	22.731	4.5462	15147.4	0.0000
Rate	3	0.454	0.1514	504.40	0.0000
Isolate*Insecticide*Rate	176	90.839	0.5161	1719.70	0.0000
Error	382	0.115	0.0003		
Total	575	199.423			
Coefficient of variance			1.39		

Table 26 Analysis of variance table by factorial treatment effects and interaction of growth inhibition of nematophagous fungi on five fungicides at 3 days after incubation

Source	df	SS	MS	F	P
Replication	2	17	9	2.04	0.1312
Isolate	7	17198	2457	582.72	0.0000
Fungicide	4	610656	152664	36208.7	0.0000
Rate	3	6381	2127	504.48	0.0000
Isolate*Fungicide*Rate	145	61400	423	100.43	0.0000
Error	318	1341	4		
Total	479	696994			
Coefficient of variance			2.85		

Table 27 Analysis of variance table by factorial treatment effects and interaction of growth inhibition of nematophagous fungi on five fungicides at 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	5	2	1.10	0.3348
Isolate	7	13345	1906	918.54	0.0000
Fungicide	4	627198	156800	75549.2	0.0000
Rate	3	8550	2850	1373.15	0.0000
Isolate*Fungicide*Rate	145	58228	402	193.49	0.0000
Error	318	660	2		
Total	479	707985			
Coefficient of variance			2.07		

Table 28 Analysis of variance table by factorial treatment effects and interaction of growth inhibition of nematophagous fungi on five fungicides at 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	20	10	0.22	0.7992
Isolate	7	10813	1545	35.49	0.0000
Fungicide	4	620616	155154	3565.15	0.0000
Rate	3	6986	2329	53.51	0.0000
Isolate*Fungicide*Rate	145	52247	360	8.28	0.0000
Error	318	13839	44		
Total	479	704521			
Coefficient of variance			9.78		

Table 29 Analysis of variance table by factorial treatment effects and interaction of growth inhibition of nematophagous fungi on five fungicides at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	13	7	7.81	0.0005
Isolate	7	9306	1329	1562.14	0.0000
Fungicide	4	725390	181348	213088	0.0000
Rate	3	14713	4904	5762.82	0.0000
Isolate*Fungicide*Rate	145	66722	460	540.69	0.0000
Error	318	271	1		
Total	479	816415			
Coefficient of variance			1.48		

Table 30 Analysis of variance table by factorial treatment effects and interaction of sporulation of nematophagous fungi on five fungicides at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.011	0.00530	13.94	0.0000
Isolate	7	67.665	9.66640	25406.0	0.0000
Fungicide	4	15.750	3.93742	10348.7	0.0000
Rate	3	1.570	0.52332	1375.43	0.0000
Isolate*Fungicide*Rate	145	50.391	0.34752	913.39	0.0000
Error	318	0.121	0.00038		
Total	479	135.507			
Coefficient of variance			1.60		

Table 31 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi on three herbicides at 3 days after incubation

Source	df	SS	MS	F	P
Replication	2	28	14.0	2.50	0.0852
Isolate	7	369179	52739.9	9424.70	0.0000
Herbicide	2	20628	10314.1	1843.14	0.0000
Rate	3	20897	6965.7	1244.77	0.0000
Isolate*Herbicide*Rate	83	95736	1153.5	206.12	0.0000
Error	190	1063	5.6		
Total	287	507532			
Coefficient of variance			7.04		

Table 32 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi on three herbicides at 5 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.35261	0.2	0.04	0.9607
Isolate	7	72479.5	10354.2	2352.78	0.0000
Herbicide	2	67909.7	33954.8	7715.52	0.0000
Rate	3	63280.0	21093.3	4793.02	0.0000
Isolate*Herbicide*Rate	83	77145.0	929.5	211.20	0.0000
Error	190	836.161	4.4		
Total	287	281651			
Coefficient of variance			2.98		

Table 33 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi on three herbicides at 7 days after incubation

Source	df	SS	MS	F	P
Replication	2	9	4.4	1.35	0.2615
Isolate	7	72988	10426.8	3200.51	0.0000
Herbicide	2	78506	39253.1	12048.8	0.0000
Rate	3	69766	23255.4	7138.25	0.0000
Isolate*Herbicide*Rate	83	89308	1076.0	330.28	0.0000
Error	190	619	3.3		
Total	287	311196			
Coefficient of variance			2.68		

Table 34 Analysis of variance table by factorial treatment effects and interaction of the growth inhibition of nematophagous fungi growth on three herbicides at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	17	8.3	1.52	0.2207
Isolate	7	84324	12046.3	2215.52	0.0000
Herbicide	2	66443	33221.7	6110.05	0.0000
Rate	3	75964	25321.3	4657.03	0.0000
Isolate*Herbicide*Rate	83	103197	1243.3	228.67	0.0000
Error	190	1033	5.4		
Total	287	330978			
Coefficient of variance			3.62		

Table 35 Analysis of variance table by factorial treatment effects and interaction of sporulation reduction of nematophagous fungi on three herbicides at 10 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0009	0.00043	4.83	0.0090
Isolate	7	4.7082	0.67260	7613.21	0.0000
Herbicides	2	5.5483	2.77417	31400.9	0.0000
Rate	3	0.0162	0.00541	61.26	0.0000
Isolate*Herbicides*Rate	83	9.5537	0.11510	1302.87	0.0000
Error	190	0.0168	0.00009		
Total	287	19.8441			
Coefficient of variance			0.86		

Table 36 Analysis of variance table by factorial treatment effects and interaction of the sporulation of nematophagous fungi on seven solid substrate media at 21 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0007	0.0004	0.97	0.3810
Solid media	6	0.5941	0.0990	267.83	0.0000
Isolate	7	71.4452	10.2065	27608.0	0.0000
Solid media*Isolate	42	3.8702	0.0921	249.25	0.0000
Error	110	0.0407	0.0004		
Total	167	75.9508			
Coefficient of variance			1.54		

Table 37 Analysis of variance table by factorial treatment effects and interaction of the fresh weight biomass of nematophagous fungi on eight liquid media at 14 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.1093	0.05465	3.73	0.0316
Media	7	28.5919	4.08455	278.48	0.0000
Isolate	2	4.1342	2.06710	140.93	0.0000
Media*Isolate	14	51.9970	3.71407	253.22	0.0000
Error	46	0.6747	0.01467		
Total	71	85.5070			
Coefficient of variance			3.77		

Table 38 Analysis of variance table by factorial treatment effects and interaction of the dry weight biomass of nematophagous fungi on eight liquid media at 14 days after incubation

Source	df	SS	MS	F	P
Replication	2	0.0102	0.00509	3.28	0.0464
Media	7	2.3906	0.34151	220.36	0.0000
Isolate	2	2.0026	1.00130	646.10	0.0000
Media*Isolate	14	10.3502	0.73930	477.04	0.0000
Error	46	0.0713	0.00155		
Total	71	14.8249			
Coefficient of variance			4.75		

Table 39 Analysis of variance table by factorial treatment effects and interaction of sporulation of nematophagous fungi on eight liquid media at 14 days after incubation

Source	df	SS	MS	F	P
Replication	9	0.0183	0.0020	0.97	0.4650
Media	7	1.7248	0.2464	117.86	0.0000
Isolate	2	38.3282	19.1641	9167.21	0.0000
Media*Isolate	14	3.4509	0.2465	117.91	0.0000
Error	207	0.4327	0.0021		
Total	239	43.9549			
Coefficient of variance			3.56		

Table 40 Analysis of variance table by two factorial treatment effects and interaction of the percentage of head lettuce seedling emergent at 7 days after planting

Source	df	SS	MS	F	$F_{0.01}$
Replication	2	110.1111	55.0556	6.28	5.39
Treatment	17	11932.8333	701.9314	80.12	2.70
A	8	5057.3333	632.1667	72.15	3.17
B	1	378.6852	378.6852	43.22	7.56
AxB	8	6496.8148	812.1019	92.69	3.17
Eroor	34	297.8889	8.7614		
Total	53	12340.8333	232.8459		
Coefficient of variance			3.94		

Table 41 Analysis of variance table by two factorial treatment effects and interaction of the percentage of head lettuce seedling emergence at 14 days after planting

Source	df	SS	MS	F	$F_{0.01}$
Replication	2	1.9259	0.9630	0.39	5.39
Treatment	17	614.7593	36.1623	14.51	2.70
A	8	346.2593	43.2824	17.37	3.17
B	1	83.1296	83.1296	33.35	7.56
AxB	8	185.3704	23.1713	9.30	3.17
Error	34	84.7407	2.4924		
Total	53	701.4259	13.2345		
Coefficient of variance			1.69		

Table 42 Analysis of variance table by two factorial treatment effects and interaction of plant height of head lettuce seedling on different fungal biomass at 30 days after planting

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	30.7981	3.4220	4.96	2.56
Treatment	17	444.3668	26.1392	37.92	2.19
A	8	166.3963	20.7995	30.18	2.66
B	1	3.0109	3.0109	4.37	6.85
AxB	8	274.9596	34.3700	49.86	2.66
Error	153	105.4623	0.6893		
Total	179	580.6272	3.2437		
Coefficient of variance			7.44		

Table 43 Analysis of variance table by two factorial treatment effects and interaction of root length of head lettuce seedling on different fungal biomass at 30 days after planting

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	11.8631	1.3181	2.38	2.56
Treatment	17	639.8056	37.6356	68.05	2.19
A	8	178.6061	22.3258	40.37	2.66
B	1	3.5617	3.5617	6.44	6.85
AxB	8	457.6378	57.2047	103.43	2.66
Error	153	84.6206	0.5531		
Total	179	736.2893	4.1133		
Coefficient of variance			7.45		

Table 44 Analysis of variance table by two factorial treatment effects and interaction of fresh weight of shoot of head lettuce seedling on different fungal biomass at 30 days after planting

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	0.1300	0.0144	2.47	2.56
Treatment	17	68.7680	4.0452	692.37	2.19
A	8	61.0846	7.6356	1306.90	2.66
B	1	0.2645	0.2645	45.27	6.85
AxB	8	7.4189	0.9274	158.73	2.66
Error	153	0.8939	0.0058		
Total	179	69.7919	0.3899		
Coefficient of variance			7.40		

Table 45 Analysis of variance table by two factorial treatment effects and interaction of dry weight of shoot of head lettuce seedling on different fungal biomass at 30 days after planting

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	0.0108	0.0012	1.11	2.56
Treatment	17	0.3774	0.0222	20.42	2.19
A	8	0.2008	0.0251	23.09	2.66
B	1	0.0405	0.0405	37.25	6.85
AxB	8	0.1361	0.0170	15.64	2.66
Error	153	0.1664	0.0011		
Total	179	0.5546	0.0031		
Coefficient of variance			23.48		

Table 46 Analysis of variance table by two factorial treatment effects and interaction of plant height of head lettuce on different fungal biomass at 60 days after transplantation in pots containing root-knot nematodes

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	71.9778	7.9975	2.01	2.56
Treatment	17	693.2611	40.7801	10.27	2.19
A	8	391.0861	48.8858	12.31	2.66
B	1	28.0056	28.0056	7.05	6.85
AxB	8	274.1694	34.2712	8.63	2.66
Error	153	607.5722	3.9711		
Total	179	1372.8111	7.6693		
Coefficient of variance			7.02		

Table 47 Analysis of variance table by two factorial treatment effects and interaction of root length of head lettuce on different fungal biomass at 60 days after transplantation in pots containing root-knot nematodes

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	24.4432	2.7159	1.49	2.56
Treatment	17	122.7371	7.2198	3.97	2.19
A	8	71.6151	8.9519	4.92	2.66
B	1	4.2936	4.2936	2.36	6.85
AxB	8	46.8284	5.8536	3.22	2.66
Error	153	278.1728	1.8181		
Total	179	425.3531	2.3763		
Coefficient of variance			9.96		

Table 48 Analysis of variance table by two factorial treatment effects and interaction of fresh weight of shoot of head lettuce on different fungal biomass at 60 days after transplantation in pots containing root-knot nematodes

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	540.7719	60.0858	0.73	2.56
Treatment	17	43243.2788	2543.7223	31.12	2.19
A	8	28957.7210	3619.7151	44.28	2.66
B	1	153.6981	153.6981	1.88	6.85
AxB	8	14131.8597	1766.4825	21.61	2.66
Error	153	12508.0783	81.7521		
Total	179	56292.1290	314.4812		
Coefficient of variance			18.33		

Table 49 Analysis of variance table by two factorial treatment effects and interaction of dry weight of shoot of head lettuce on different fungal biomass at 60 days after transplantation in pots containing root-knot nematodes

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	1.2347	0.1372	0.54	2.56
Treatment	17	224.7133	13.2184	52.40	2.19
A	8	84.9792	10.6224	42.11	2.66
B	1	0.6468	0.6468	2.56	6.85
AxB	8	139.0873	17.3859	68.92	2.66
Error	153	38.5939	0.2522		
Total	179	264.5419	1.4779		
Coefficient of variance			15.47		

Table 50 Analysis of variance table by two factorial treatment effects and interaction of gall per root of head lettuce on different fungal biomass at 60 days after transplantation in pots containing root-knot nematodes

Source	df	SS	MS	F	$F_{0.05}$
Replication	9	30730.9167	3414.5463	10.11	1.96
Treatment	17	11830.0500	695.8853	2.06	1.75
A	8	5471.9000	683.9875	2.02	2.08
B	1	113.6056	113.6056	0.34	3.92
AxB	8	6244.5444	780.5681	2.31	2.08
Error	153	51688.7833	337.8352		
Total	179	94249.7500	526.5349		
Coefficient of variance			83.23		

Table 51 Analysis of variance table by two factorial treatment effects and interaction of % galled reduction of head lettuce on different fungal biomass at 60 days after transplantation in pots containing root-knot nematodes

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	242523.3204	26947.0356	9.42	2.56
Treatment	15	91886.7504	6125.7834	2.14	2.19
A	7	54892.0922	7841.7275	2.74	2.79
B	1	4297.3862	4297.3862	1.50	6.85
AxB	7	32697.2720	4671.0389	1.63	2.79
Error	135	386277.1976	2861.3126		
Total	159	720687.2684	4532.6243		
Coefficient of variance			151.33		

Table 52 Analysis of variance table by two factorial treatment effects and interaction of plant height of head lettuce on different biological agent (nematophagous fungi) application compared fermented and without fermented method 7 days before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	83.9668	9.3296	1.71	2.56
Treatment	17	727.3823	42.7872	7.82	2.19
A	8	326.2128	40.7766	7.46	2.66
B	1	64.6800	64.6800	11.83	6.85
AxB	8	336.4894	42.0612	7.69	2.66
Error	153	836.6182	5.4681		
Total	179	1647.9673	9.2065		
Coefficient of variance			9.56		

Table 53 Analysis of variance table by two factorial treatment effects and interaction of root length of head lettuce on different biological agent (nematophagous fungi) application compared fermented and without fermented method 7 days before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	22.7726	2.5303	1.24	2.56
Treatment	17	326.6984	19.2176	9.40	2.19
A	8	227.3314	28.4164	13.90	2.66
B	1	5.2702	5.2702	2.58	6.85
AxB	8	94.0968	11.7621	5.75	2.66
Error	153	312.7354	2.0440		
Total	179	662.2064	3.6995		
Coefficient of variance			10.54		

Table 54 Analysis of variance table by two factorial treatment effects and interaction of fresh weight of shoot of head lettuce on different biological agent (nematophagous fungi) application compared fermented and without fermented method 7 days before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	210.8899	23.4322	2.15	2.56
Treatment	17	1388.4057	81.6709	7.50	2.19
A	8	745.2024	93.1503	8.55	2.66
B	1	76.1020	76.1020	6.99	6.85
AxB	8	567.1014	70.8877	6.51	2.66
Error	153	1666.4906	10.8921		
Total	179	3265.7863	18.2446		
Coefficient of variance			14.20		

Table 55 Analysis of variance table by two factorial treatment effects and interaction of dry weight of shoot of head lettuce on different biological agent (nematophagous fungi) application comparing fermented and without fermented method 7 days before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	0.7834	0.0870	0.95	2.56
Treatment	17	44.2206	2.6012	28.48	2.19
A	8	25.6331	3.2041	35.08	2.66
B	1	0.0271	0.0271	0.30	6.85
AxB	8	18.5604	2.3200	25.40	2.66
Error	153	13.9747	0.0913		
Total	179	58.9787	0.3295		
Coefficient of variance			16.93		

Table 56 Analysis of variance table by two factorial treatment effects and interaction of gall per root of head lettuce on different biological agent (nematophagous fungi) application compared fermented and without fermented method 7 days before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	717.0056	79.6673	1.05	2.56
Treatment	17	35439.7611	2084.6918	7.46	2.19
A	8	26068.1111	3258.5139	42.93	2.66
B	1	15.6056	15.6056	0.21	6.85
AxB	8	9356.0444	1169.5056	15.41	2.66
Error	153	11613.2944	75.9039		
Total	179	47770.0611	266.8718		
Coefficient of variance			58.36		

Table 57 Analysis of variance table by two factorial treatment effects and interaction of % galled reduction of head lettuce on different biological agent (nematophagous fungi) application compared fermented and without fermented method 7 days before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	2388.7718	265.4191	1.06	2.56
Treatment	15	44501.8898	2966.7927	11.83	2.19
A	7	38224.6506	5460.6644	21.77	2.79
B	1	295.3379	295.3379	1.18	6.85
AxB	7	5981.9013	854.5573	3.41	2.79
Error	135	33859.4468	250.8107		
Total	159	80750.1084	507.8623		
Coefficient of variance			20.37		

Table 58 Analysis of variance table by two factorial treatment effects and interaction of plant height of baby cos lettuce on different biological agent (nematophagous fungi) application comparing fermented and without fermented method before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	77.8903	8.6545	1.26	2.56
Treatment	17	806.3125	47.4301	6.91	2.19
A	8	537.5500	67.1937	9.80	2.66
B	1	8.2347	8.2347	1.20	6.85
AxB	8	260.5278	32.5660	4.75	2.66
Error	153	1049.5347	6.8597		
Total	179	1933.7375	10.8030		
Coefficient of variance			9.43		

Table 59 Analysis of variance table by two factorial treatment effects and interaction of root length of baby cos lettuce on different biological agent (nematophagous fungi) application comparing fermented and without fermented method before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	19.7722	2.1969	0.45	2.56
Treatment	17	294.2000	17.3059	3.52	2.19
A	8	119.4750	14.9344	3.04	2.66
B	1	0.0056	0.0056	0.00	6.85
AxB	8	174.7194	21.8399	4.45	2.66
Error	153	751.5778	4.9123		
Total	179	1065.5500	5.9528		
Coefficient of variance			14.50		

Table 60 Analysis of variance table by two factorial treatment effects and interaction of fresh weight of shoot of baby cos on different biological agent (nematophagous fungi) application comparing fermented and without fermented method before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	533.5609	59.2845	0.98	2.56
Treatment	17	9470.2068	557.0710	9.17	2.19
A	8	4443.0581	555.3823	9.14	2.66
B	1	85.7394	85.7394	1.41	6.85
AxB	8	4941.4093	617.6762	10.17	2.66
Error	153	9294.1300	60.7459		
Total	179	19297.8977	107.8095		
Coefficient of variance			21.31		

Table 61 Analysis of variance table by two factorial treatment effects and interaction of dry weight of shoot of baby cos lettuce on different biological agent (nematophagous fungi) application comparing fermented and without fermented method before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	4.7612	0.5290	1.72	2.56
Treatment	17	57.5718	3.3866	11.02	2.19
A	8	26.3229	3.2904	10.71	2.66
B	1	0.9331	0.9331	3.04	6.85
AxB	8	30.3157	3.7895	12.33	2.66
Error	153	47.0130	0.3073		
Total	179	109.3460	0.6109		
Coefficient of variance			20.31		

Table 62 Analysis of variance table by two factorial treatment effects and interaction of gall per root of baby cos lettuce on different biological agent (nematophagous fungi) application comparing fermented and without fermented method before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	4578.3611	508.7068	2.90	2.56
Treatment	17	17481.0278	1028.2958	5.85	2.19
A	8	12642.8778	1580.3597	8.99	2.66
B	1	638.4500	638.4500	3.63	6.85
AxB	8	4199.7000	524.9625	2.99	2.66
Error	153	26882.1389	175.7003		
Total	179	48941.5278	273.4164		
Coefficient of variance			118.99		

Table 63 Analysis of variance table by two factorial treatment effects and interaction of % galled reduction of baby cos lettuce on different biological agent (nematophagous fungi) application comparing fermented and without fermented method before transplantation

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	77768.6167	8640.9574	2.54	2.56
Treatment	15	277419.2214	18494.6148	5.44	2.19
A	7	236254.1449	33750.5921	9.93	2.79
B	1	14476.5029	14476.5029	4.26	6.85
AxB	7	26688.5735	3812.6534	1.12	2.79
Error	135	459041.0011	3400.3037		
Total	159	814228.8391	5120.9361		
Coefficient of variance			101.34		

Table 64 Analysis of variance table by two factorial treatment effects and interaction of plant height of head lettuce by nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	18.7506	2.0834	0.99	2.56
Treatment	17	2142.6578	126.0387	59.91	2.19
A	8	1704.0518	213.0065	101.25	2.66
B	1	0.0376	0.0376	0.02	6.85
AxB	8	438.5684	54.8211	26.06	2.66
Error	153	321.8614	2.1037		
Total	179	2483.2698	13.8730		
Coefficient of variance			11.55		

Table 65 Analysis of variance table by two factorial treatment effects and interaction of root length of head lettuce by nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	15.2222	1.6914	1.37	2.56
Treatment	17	2303.4278	135.4958	109.47	2.19
A	8	2274.4278	284.3035	229.69	2.66
B	1	8.4500	8.4500	6.83	6.85
AxB	8	20.5500	2.5688	2.08	2.66
Error	153	189.3778	1.2378		
Total	179	2508.0278	14.0113		
Coefficient of variance			8.80		

Table 66 Analysis of variance table by two factorial treatment effects and interaction of fresh weight of shoot of head lettuce by nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	1171.0985	130.1221	0.48	2.56
Treatment	17	249729.5420	14689.9731	54.72	2.19
A	8	158350.8417	19793.8552	73.74	2.66
B	1	1526.7618	1526.7618	5.69	6.85
AxB	8	89851.9385	11231.4923	41.84	2.66
Error	153	41071.9294	268.4440		
Total	179	291972.5700	1631.1317		
Coefficient of variance			13.44		

Table 67 Analysis of variance table by two factorial treatment effects and interaction of dry weight of shoot of head lettuce by nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	3.7969	0.4219	0.46	2.56
Treatment	17	598.4047	35.2003	38.16	2.19
A	8	398.8727	49.8591	54.05	2.66
B	1	0.0654	0.0654	0.07	6.85
AxB	8	199.4667	24.9333	27.03	2.66
Error	153	141.1452	0.9225		
Total	179	743.3468	4.1528		
Coefficient of variance			14.38		

Table 68 Analysis of variance table by two factorial treatment effects and interaction of gall per root of head lettuce by nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	1149.0056	127.6673	1.24	2.56
Treatment	17	11346.6278	667.4487	6.48	2.19
A	8	5480.8778	685.1097	6.65	2.66
B	1	28.0056	28.0056	0.27	6.85
AxB	8	5837.7444	729.7181	7.09	2.66
Error	153	15757.0944	102.9875		
Total	179	28252.7278	157.8365		
Coefficient of variance			46.63		

Table 69 Analysis of variance table by two factorial treatment effects and interaction of % galled reduction of head lettuce by nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area

Source	df	SS	MS	F	$F_{0.01}$
Replication	9	6918.7453	768.7495	1.56	2.56
Treatment	15	21628.1160	1441.8744	1.75	2.19
A	7	11107.6774	1586.8111	3.22	2.79
B	1	52.4410	52.4410	0.11	6.85
AxB	7	10467.9976	1495.4282	3.03	2.79
Error	135	66561.3592	493.0471		
Total	159	95108.2206	598.1649		
Coefficient of variance			42.78		

Table 70 Analysis of variance table by RCBD of plant height of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 1)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	9.0500	1.0056	1.12	2.04	2.72	0.3553
Treatment	9	199.2500	22.1389	24.75	2.04	2.72	0.0000
Ex.Error	81	72.4500	0.8944				
Total	99	280.7500	2.8359				
Coefficient of variance				4.71			

Table 71 Analysis of variance table by RCBD of root length of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 1)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	23.7625	2.6403	0.80	2.04	2.72	0.6219
Treatment	9	128.8125	14.3125	4.32	2.04	2.72	0.0003
Ex.Error	81	268.6125	3.3162				
Total	99	421.1875	4.2544				
Coefficient of variance				17.38			

Table 72 Analysis of variance table by RCBD of fresh weight of shoot of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 1)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	43625.0000	4847.2222	1.39	2.04	2.72	0.2049
Treatment	9	649225.0000	72136.1111	20.72	2.04	2.72	0.0000
Ex.Error	81	282025.0000	3481.7901				
Total	99	974875.0000	9847.2222				
Coefficient of variance				22.73			

Table 73 Analysis of variance table by RCBD of dry weight of shoot of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 1)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	63.6571	7.0730	1.43	2.04	2.72	0.1873
Treatment	9	326.6813	36.2979	7.36	2.04	2.72	0.0000
Ex.Error	81	399.6367	4.9338				
Total	99	789.9751	7.9795				
Coefficient of variance				23.97			

Table 74 Analysis of variance table by RCBD of gall per root of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 1)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	185.3600	20.5956	0.29	2.04	2.72	0.9761
Treatment	9	18858.9600	2095.4400	29.16	2.04	2.72	0.0000
Ex.Error	81	5821.4400	71.8696				
Total	99	24865.7600	251.1693				
Coefficient of variance				90.96			

Table 75 Analysis of variance table by RCBD of gall per root of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 1)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	1883.7537	209.3060	0.77	2.04	2.72	0.6475
Treatment	8	10115.1473	1264.3934	4.64	2.09	2.82	0.0003
Ex.Error	72	19627.7938	272.6082				
Total	89	31626.6947	355.3561				
Coefficient of variance				18.44			

Table 76 Analysis of variance table by RCBD of plant height of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 2)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	12.6000	1.4000	1.27	2.04	2.72	0.2646
Treatment	9	102.2000	11.3556	10.31	2.04	2.72	0.0000
Ex.Error	81	89.2000	1.1012				
Total	99	204.0000	2.0606				
Coefficient of variance				4.95			

Table 77 Analysis of variance table by RCBD of root length of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 2)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	17.4400	1.9378	0.95	2.04	2.72	0.5086
Treatment	9	83.2900	9.2544	4.52	2.04	2.72	0.0002
Ex.Error	81	165.9600	2.0489				
Total	99	266.6900	2.6938				
Coefficient of variance				14.18			

Table 78 Analysis of variance table by RCBD of fresh weight of shoot of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 2)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	20208.6000	2245.4000	1.45	2.04	2.72	0.1790
Treatment	9	221864.4000	24651.6000	15.96	2.04	2.72	0.0000
Ex.Error	81	125073.0000	1544.1111				
Total	99	367146.0000	3708.5455				
Coefficient of variance				29.58			

Table 79 Analysis of variance table by RCBD of dry weight of shoot of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 2)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	45.1996	5.0222	0.93	2.04	2.72	0.5012
Treatment	9	439.2180	48.8020	9.08	2.04	2.72	0.0000
Ex.Error	81	435.5342	5.3770				
Total	99	919.9518	9.2924				
Coefficient of variance				35.18			

Table 80 Analysis of variance table by RCBD of gall per root of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 2)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	544.4900	60.4989	1.23	2.04	2.72	0.2883
Treatment	9	12331.4900	1370.1656	27.84	2.04	2.72	0.0000
Ex.Error	81	3985.8100	49.2075				
Total	99	16861.7900	170.3211				
Coefficient of variance				77.00			

Table 81 Analysis of variance table by RCBD of % galled reduction of head lettuce by bio-formulations of nematophagous fungi at 60 days after transplantation in root-knot nematode infested area (Area 2)

Source	df	SS	MS	F	F.05	F.01	F-Prob
Block	9	2541.3617	282.3735	1.30	2.04	2.72	0.2504
Treatment	8	1768.9470	221.1184	1.02	2.09	2.82	0.4298
Ex.Error	72	15613.1963	216.8499				
Total	89	19923.5050	223.8596				
Coefficient of variance				16.92			

Table 82 Source of the nutritional component on seven solid substrate media and their cost

Media	Source					Cost of raw component (Baht/kg)
	Carbon	**Nitrogen**	**Phosphorus**	**Trace elements**	**Growth factors**	
Corn	fiber, fat, carbohydrates, sugar	protein	phosphorus	Mg, Ca, Mn, Fe, Zn, Na, Cu, Se	niacin, thiamin, folate, riboflavin, pantothenic acid , beta-carotene, **lutein+zeazanthi**[1/], vitamin A, B6, C, E, K	14
Soybean	fiber, **fat**, carbohydrates	protein	phosphorus	Mg, **Ca**, Mn, Fe, Zn, Na, Cu, Se	niacin, thiamin, folate, **riboflavin**, pantothenic acid, vitamin A, B6 **, C**	70
Glutinous Rice	fiber, fat, **carbohydrates**, sugar	protein	phosphorus	Mg, Ca, Mn, Fe, Zn, Na, Cu, Se	niacin, **thiamin**, **folate**, riboflavin, **pantothenic acid** , vitamin B6, E, K	36
Rice	fiber, fat, **carbohydrates**, sugar	protein	phosphorus	Mg, Ca, Mn, Fe, Zn, Na, Cu, Se	niacin, **thiamin**, **folate**, riboflavin, **pantothenic acid** , vitamin B6, E, K	25
Rice mixed V8 juice	fiber, fat, **carbohydrates**, sugar	protein	phosphorus	Mg, Ca, Mn, Fe, Zn, Na, Cu, Se	niacin, **thiamin**, **folate**, riboflavin, **pantothenic acid** , vitamin A, B6, C, E, K	41
Corn mixed V8 juice	fiber, fat, carbohydrates, sugar	protein	phosphorus	Mg, Ca, Mn, Fe, Zn, Na, Cu, Se	niacin, thiamin, folate, riboflavin, pantothenic acid , beta-carotene, **lutein+zeazanthi**, vitamin A, B6, C, E, K	30
Millet	fiber, fat, carbohydrates	protein	phosphorus	Mg, Ca, Mn, Fe, Zn	tryptophan, niacin, B6, thiamin, folate, riboflavin	18

Source: Wikipedia (2012h)

[1]/ bold letter = high amount

Table 83 The nutritional sources of eight liquid media and their cost

Media	Source					Cost
	Carbon	Nitrogen	Phosphorus	Trace elements	Growth factors	(Baht/lit.)
½ adamek-lösung	agar, malt	V8	V8	V8, $CaCo_3$, agar	yeast extract, V8	107.7
Beef corn	sucrose, corn powder	corn powder	corn powder	$CaCo_3$	beef extract	406.80
Coconut	coconut milk	protein hydrolysis	coconut milk	coconut milk	coconut milk	7.18
Egg corn	sucrose, corn powder	egg yolk	egg yolk	egg yolk	egg yolk	2.70
Enteromophthoraceae	agar, malt extract	V8	V8	V8, $CaCo_3$, agar	V8	64.26
Gemüsesaft-malzextrakt	glucose	corn steep liquor			yeast extract, corn steep liquor	119.86
V8 rice	sucrose, rice powder	corn steep liquor	V8	V8	V8, corn steep liquor	8.41
V8	V8	V8	V8	V8, $CaCo_3$	V8	32.79

Table 84 Useful nematode related websites

Subject	Website
Identification of nematodes:	http://nematode.unl.edu/key/nemakey.htm
Nematodes feeding:	http://www.cropsci.uiuc.edu/faculty/lambert-k/
SCN mating:	http://www.cropsci.uiuc.edu/faculty/lambert-k/
Nematodes movies:	http://www.cropsci.uiuc.edu/faculty/lambert-k/
Phylogenetic trees of nematodes:	http://www.wormbook.org/chapters/www_quicktourdiversity/quicktourdiversity.html
University of Nebraska – Lincoln Nematology:	http://nematode.unl.edu/
Iowa State University, Soybean cyst nematode:	http://nematode.unl.edu/scn/scnisu.htm
Alternative nematode control:	http://www.attra.org/attra-pub/nematode.html
Soybean cyst nematode:	http://extension.missouri.edu/xplor/agguides/crops/g04450.htm
Nematode identification:	http://kbn.ifas.ufl.edu/gaster/identify.htm
The virtual nematode:	http://www.ppws.vt.edu/~sforza/nematode.html
Other nematodes:	http://elegans.swmed.edu/Nematodes/
UC Davis Nematode databases:	http://ucdnema.ucdavis.edu/search.html
Society of nematologists:	http://www.nematologists.org/
C. elegans information:	http://elegans.swmed.edu/
Biocontrol web site:	http://sacs.cpes.peachnet.edu/nemabc/
History of Nematology:	http://flnem.ifas.ufl.edu/nemadoc.htm
Chemical methods of nematode control:	http://plpnemweb.ucdavis.edu/nemaplex/Mangmnt/Chemical.htm

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Education

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Conferences/Workshops

1. **Certificate of Teaching**: Multicountry Observational Study Mission on Advanced Technologies for Greenhouse Farming. Asian Productivity Organization (APO) Project, Seoul, Republic of Korea, 16–20 February 2009.

2. **Certificate of Attendance**: AGRIA International Conference. Le Meridien Hotel, Chiang Mai, Thailand, 3-4 December 2010.

3. **Certificate of Teaching**: Workshop on Management Systems for Safe, Reliable, High-quality Food and Agricultural Products. Asian Productivity Organization (APO) Project, Tokyo and Sendai, Japan, 26–30 March 2012.

PROCEEDING AND PUBLICATIONS

The 3rd Annual Meeting of Thai Mycological Association and
Mycology Conference in Thailand. 11 October 2008, p. 50.

Screening and Production of Antagonistic Fungi (*Arthrobotrys* sp.) for Controlling Root Knot Nematode in Head Lettuce

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Abstract: Dual culture tests between pairs of three antagonistic fungi i.e. *Arthrobotrys* spp. (8 isolates), *P. lilacinus* and *T. harzianum*, showed that *T. harzianum* grew more quickly and its mycelium inhibited growth of *Arthrobotrys* spp. by invading and covering the *Arthrobotrys* spp. 's colonies. No binding or penetration of the hyphae of the two fungi on the hyphae of *Arthrobotrys* spp. was found. But the areas where *T. harzianum* grew on top of *Arthrobotrys* spp.'s colonies had no spore production. *T. harzianum* grew faster than *P. lilacinus* and inhibited growth of the later as well. A test on capability of *Arthrobotrys* spp. to capture the J2 (root knot nematode juvenile stage 2) was carried out on diluted animal feed corn medium. It was found that Dong con isolate showed best result while HNR oli, PD and Dong oli came second, third and fourth respectively. A pot test; comparison of capability of 4 selected isolates and carbofuran to control root knot nematode in head lettuce by mixing with the soil before planting. Results showed that Dong con and Dong oli isolates could reduce root knot numbers, increase fresh weight of the plant and reduce population of J2 but their efficacy was lower than carbofuran. A study on finding a suitable compost to multiply the *Arthrobotrys* spp. fungus: the compost consisted of 50 % of cow dung manure, 20 % of ash, 10 % of rice bran, 10 % of rice husk and 10 % of coconut peat with 15 days fermentation showed highest sporulation. The proportion of *Arthrobotrys* spp. in the compost to mix with soil at the rate of 1:2 (300 g / 600 g soil), could reduce J2 population most.

Key words: biological control, Meloidogyne, root knot nematode, nematophagous fungi, *Arthrobotrys*

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การคัดเลือกและทดสอบประสิทธิภาพเชื้อราปฏิปักษ์ไส้เดือนฝอยรากปมในพืชผักบนพื้นที่สูง

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บทคัดย่อ

ผลการศึกษาและทดสอบประสิทธิภาพเชื้อราปฏิปักษ์ไส้เดือนฝอยรากปม *Meloidogyne incognita* ในห้องปฏิบัติการเพื่อพัฒนาเป็นชีวภัณฑ์กำจัดไส้เดือนฝอยรากปมพืชผัก พบว่า ตัวอย่างดินร่วนปนทรายที่เก็บจากแปลงปลูกผักกาดหอมห่อ เบบี้คอส ยาสูบ มะเขือเทศ พริก บีทรูท แครอท และแตงกวาญี่ปุ่น ซึ่งมีประวัติการแพร่ระบาดไส้เดือนฝอยรากปม รวม 45 แปลง ในพื้นที่ 4 จังหวัด ได้แก่ เชียงใหม่ 36 ตัวอย่าง เชียงราย 2 ตัวอย่าง นครสวรรค์ 1 ตัวอย่าง และแม่ฮ่องสอน 6 ตัวอย่าง สามารถแยกเชื้อราปฏิปักษ์ฯ ด้วย soil scattering method บนอาหาร Water agar ที่หยดด้วยสารแขวนลอยตัวอ่อนไส้เดือนฝอยรากปม *M. incognita* ระยะที่ 2 (J2) ความเข้มข้น 100 ตัวต่อซ้ำ พบเชื้อราปฏิปักษ์ฯ จำนวน 116 ไอโซเลท แบ่งเป็น 6 ตระกูล ตามลักษณะทางสัณฐานวิทยา คือ *Arthrobotrys oligospora* (72%), *Arthrobotrys conoides* (5%), *Arthrobotrys musiformis* (5%), *Monacrosporium thaumasium* (10%), *Paecilomyces lilacinus* (5%) และ *Pochonia chlamydosporia var. catenulate* (3%) สำหรับการทดสอบประสิทธิภาพการกำจัดตัวอ่อน (J2) และไข่ไส้เดือนฝอยรากปมบนอาหาร WA สามารถคัดเลือกเชื้อราฯ ที่มีเปอร์เซ็นต์การกำจัด J2 สูงสุดหลังจาก 7 วัน จำนวน 6 ไอโซเลท 80-90% และกำจัดไข่ 2 ไอโซเลท 70-75% อย่างไรก็ตาม รูปแบบห่วง (trap) หรือกระบวนการกำจัด J2 และไข่ของเชื้อราแต่ละตระกูลจะแตกต่างกัน นอกจากนี้ยังพบว่า เชื้อรา *Arthrobotrys musiformis* สามารถผลิตกลิ่นเฉพาะคล้ายแอมโมเนียได้ด้วย

คำสำคัญ: เชื้อราปฏิปักษ์ไส้เดือนฝอย *Meloidogyne incognita* รากปม

การคัดเลือกและทดสอบประสิทธิภาพเชื้อราปฏิปักษ์ใส้เดือนฝอยรากปมในพืชผักบนพื้นที่สูง

Screening and Pre-test of Antagonistic Fungi Efficiency for Controlling Root-knot Nematode on Highland Vegetable Productions

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Abstract

Results from a study and tests on antagonistic fungi efficiency against root-knot nematode, *Meloidogyne incognita*, carried out in the laboratory to develop bio-product controlling root-knot nematode showed that from samples collected from sandy loam from 45 vegetable plots growing head lettuces, baby cos, lettuces, tobaccos, tomatoes, chilies , beetroots, carrots, and Japanese cucumbers, all of which have records on the spread of root-knot nematode and are in 4 provinces including Chiang Mai (36 samplings) Chiang Rai (2 samplings) Nakornsawan (1 sampling) and Mae Hong Son (6 samplings), antagonistic fungi could be isolated by soil scattering method. On Water agar, when dripping suspended solid containing second stage juveniles (J2) of root-knot nematode *M. incognita* with intensity of 100, 116 fungal isolates were found and could be morphologically classified into 3 families including: *Arthrobotrys spp.* (82%) *Monacrosporium* spp. (10%) *Paecilomyces* spp. (5%) and Unknown (3%). Concerning efficiency of elimination of the second stage juveniles (J2) and egg masses of root-knot nematodes on WA , the results found were that after 7 days, 8 antagonistic fungal ; DLo 001 and MTo 010 (*Arthrobotrys oligospora*) MSr 018 (*Arthrobotrys conoides*) MSr 007 (*Arthrobotrys musiformis*) JDl 001 and MPa 007 (*Monacrosporium thaumasium*) WJ 003 *Paecilomyces lilacinus* and MPl 007 Unknown isolates with the highest rate of J2 elimination (80-90%) and 70-75% with the highest rate of egg masses elimination were screened. However, trap formations or J2 and egg masses elimination process of each family were different. In addition, it was found that *Arthrobotrys musiformis* can produce a certain ammonia-like odor.

Keywords: antagonistic fungi against nematodes, *Meloidogyne incognita*, root-knot

บทคัดย่อ

ผลการศึกษาและทดสอบประสิทธิภาพเชื้อราปฏิปักษ์ไส้เดือนฝอยรากปม *Meloidogyne incognita* ในห้องปฏิบัติการเพื่อพัฒนาเป็นชีวภัณฑ์กำจัดไส้เดือนฝอยรากปมพืชผัก พบว่า ตัวอย่างดินร่วนปนทรายที่เก็บจากแปลงปลูกผักกาดหอมห่อ เบบี้คอส ยาสูบ มะเขือเทศ พริก บีทรูท แครอท และแตงกวาญี่ปุ่น ซึ่งมีประวัติการแพร่ระบาดไส้เดือนฝอยรากปม รวม 45 แปลง ในพื้นที่ 4 จังหวัด ได้แก่ เชียงใหม่ 36 ตัวอย่าง เชียงราย 2 ตัวอย่าง นครสวรรค์ 1 ตัวอย่าง และแม่ฮ่องสอน 6 ตัวอย่าง สามารถแยกเชื้อราปฏิปักษ์ฯ ด้วย soil scattering method บนอาหาร Water agar ที่หยดด้วยสารแขวนลอยตัวอ่อนไส้เดือนฝอยรากปม *M. incognita* ระยะที่ 2 (J2) ความเข้มข้น 100 ตัว ต่อซ้ำ พบ เชื้อราปฏิปักษ์ฯ จำนวน 116 ไอโซเลท แบ่งเป็น 3 ตระกูล ตามลักษณะทางสัณฐานวิทยา คือ *Arthrobotrys* spp. (ร้อยละ 82) *Monacrosporium* spp. (ร้อยละ 10) *Paecilomyces* spp. (ร้อยละ 5) และ Unknown (ร้อยละ 3) สำหรับการทดสอบประสิทธิภาพการกำจัดตัวอ่อน (J2) และไข่ไส้เดือนฝอยรากปม บนอาหาร WA สามารถคัดเลือกเชื้อราฯ ที่มีเปอร์เซ็นต์การกำจัด J2 และไข่ สูงสุดหลังจาก 7 วัน จำนวน 8 ไอโซเลท ได้แก่ ไอโซเลท DLo 001 และ MTo 010 (*Arthrobotrys oligospora*) MSr 018 (*Arthrobotrys conoides*) MSr 007 (*Arthrobotrys musiformis*) JDl 001 และMPa 007 (*Monacrosporium thaumasium*) WJ 003 *Paecilomyces lilacinus* และ MPl 007 Unknown โดยระดับการทำลาย J2 มีค่าร้อยละ 80-90 และทำลายไข่ ร้อยละ 70-75 อย่างไรก็ตามรูปแบบห่วง (trap) หรือกระบวนการกำจัด J2 และไข่ ของเชื้อราแต่ละตระกูลจะแตกต่างกัน นอกจากนี้ยังพบว่า เชื้อรา *Arthrobotrys musiformis* สามารถผลิตกลิ่นเฉพาะคล้ายแอมโมเนียได้ด้วย

คำสำคัญ: เชื้อราปฏิปักษ์ไส้เดือนฝอย, *Meloidogyne incognita*, รากปม

คำนำ

เกษตรกรบนพื้นที่สูงนิยมปลูกพืชผัก เช่น ผักกาดหอมห่อ ผักกาดหวาน มะเขือเทศ พริกหวาน และพืชเมืองหนาว เพื่อจำหน่ายสร้างรายได้ให้กับครัวเรือน แต่เนื่องจากพื้นที่สูงส่วนใหญ่ เป็นแหล่งต้นน้ำและความหลากหลายทางชีวภาพ ในขณะเดียวกันเกษตรกรต้องปฏิบัติตามข้อกำหนดการใช้ประโยชน์ที่ดินเพื่อการเกษตรทำให้ต้องเพาะปลูกบนพื้นที่เดิมส่งผลให้เกิดการสะสมโรคและแมลงศัตรูพืชในดิน โดยไส้เดือนฝอยรากปมเป็นศัตรูพืชสำคัญชนิดหนึ่งที่สร้างความเสียหายให้กับพืชผักตระกูล Solanaceae compositae และCruciferae ทำให้น้ำหนักต้นสดลดลง 10-70 เปอร์เซ็นต์ ทั้งนี้ขึ้นอยู่กับจำนวนประชากร

ไส้เดือนฝอยรากปมในดิน สำหรับวิธีการป้องกันกำจัดไส้เดือนฝอยนิยมปัจจุบัน คือ การใช้สารเคมีอบดิน การปลูกปอเทืองหรือดาวเรืองที่มีคุณสมบัติไล่ไส้เดือนฝอย การไขน้ำท่วมแปลง และการใช้เชื้อราปฏิปักษ์ อย่างไรก็ตามสภาพภูมิสังคม ภูมิประเทศบนพื้นที่สูง ภูมิอากาศที่แปรปรวนตลอดปี และนโยบายมาตรฐานอาหารปลอดภัยเป็นข้อจำกัดหลักในการคัดเลือกวิธีการแก้ไขที่อาจต้องเฉพาะกับสภาพบนพื้นที่สูง ซึ่งการใช้เชื้อจุลินทรีย์มีแนวโน้มเหมาะสมที่สุด ด้วยเหตุนี้จึงรวบรวม คัดเลือกและทดสอบเชื้อราปฏิปักษ์ที่มีประสิทธิภาพในการควบคุมไส้เดือนฝอยรากปม (*Meloidogyne incognita*) สามารถปรับตัวให้มีชีวิตอยู่รอดบนพื้นที่สูงเพื่อพัฒนาต่อเนื่องเป็นชีวภัณฑ์ในระยะต่อไป

อุปกรณ์และวิธีการ

1. เก็บรวบรวมและแยกเชื้อราปฏิปักษ์ไส้เดือนฝอยรากปม

สำรวจแหล่งปลูกพืชบนพื้นที่สูงที่มีประวัติการแพร่ระบาดของไส้เดือนฝอยรากปม พร้อมเก็บรวบรวมตัวอย่างดินบริเวณ rhizospere ตามวิธีของ Michael and John (2006) จากนั้นแยกเชื้อราปฏิปักษ์ไส้เดือนฝอยด้วย soil scattering method บนอาหาร Water agar (WA) ที่หยดด้วยสารแขวนลอยตัวอ่อนไส้เดือนฝอยรากปม *Meloidogyne incognita* ระยะที่ 2 (J2) และไข่ สำหรับใช้เป็นเหยื่อล่อ จำนวน 100 ตัว/ฟอง ต่อจาน ทำ 6 ซ้ำ ตรวจหาเชื้อราทำลายตัวอ่อนไส้เดือนฝอยและไข่ หลังจากนั้น 3 วัน และ 5 วัน ด้วยกล้องจุลทรรศน์สเตอริโอ โดยใช้เข็มเขี่ยฆ่าเชื้อแตะสปอร์เชื้อราเพื่อนำไปแยกให้บริสุทธิ์บนอาหาร WA จากนั้นตัดปลายเส้นใยไปเลี้ยงบนอาหาร Potato Dextros Agar (PDA) เพื่อจำแนกกลุ่มตามลักษณะทางสัณฐานวิทยา (MycoBank, 2004 และ Nematophagous Fungi, 2002)

2. ทดสอบประสิทธิภาพการทำลาย J2 และไข่ ในสภาพห้องปฏิบัติการ

เลี้ยงเชื้อราปฏิปักษ์ไส้เดือนฝอยที่แยกได้แต่ละไอโซเลท บนอาหาร WA เมื่อครบ 7 วัน แบ่งหยดสารแขวนลอย J2 จำนวน 100 ตัวต่อจาน และไข่ จำนวน 100 ฟองต่อจาน อย่างละ 3 ซ้ำ โดยเตรียมสารแขวนลอย J2 และไข่ ตามวิธีการของ Jame (2004) ตรวจสอบและบันทึกผลจำนวน J2 ที่มีชีวิต และไข่ที่ฟักเป็นตัวอ่อนทุก 3 วัน 5 วัน และ7 วัน พร้อมคัดเลือกไอโซเลทเชื้อราที่มีประสิทธิภาพสูงในการกำจัด J2 และไข่ไส้เดือนฝอยรากปม

3. บันทึกข้อมูลพื้นฐานหรือลักษณะเฉพาะเชื้อราประสิทธิภาพสูงที่ถูกคัดเลือกแต่ละไอโซเลท ประกอบด้วย รูปแบบการเข้าทำลายไส้เดือนฝอยและไข่ เช่น ลักษณะห่วง ขนาด ลักษณะการกระจาย หรือโครงสร้างเส้นใย เปรียบเทียบกับแหล่งข้อมูลอ้างอิง รวมทั้งระยะเวลาที่เชื้อราเริ่มทำลายหลังจากใส่เหยื่อล่อ

ผลและวิจารณ์

1. การเก็บรวบรวมและแยกเชื้อราปฏิปักษ์ไส้เดือนฝอยรากปม

ผลสำรวจและแยกเชื้อราปฏิปักษ์ไส้เดือนฝอยจากแหล่งปลูกพืชที่มีประวัติการแพร่ระบาดของไส้เดือนฝอยรากปมบนพื้นที่สูง 4 แห่ง ได้แก่ เชียงใหม่ เชียงราย แม่ฮ่องสอน และนครสวรรค์ พบเชื้อรา จำนวน 116 ไอโซเลท แบ่งเป็น พื้นที่เชียงใหม่ 36 ตัวอย่าง เชียงราย 2 ตัวอย่าง นครสวรรค์ 1 ตัวอย่าง และแม่ฮ่องสอน 6 ตัวอย่าง จำแนกตามลักษณะทางสัณฐานวิทยาได้เป็น 3 ตระกูล คือ *Arthrobotrys* spp. พบมากที่สุด ร้อยละ 82 โดย conidia รูปร่างคล้ายผลน้ำเต้า (obovoidal to ellipsoidal, elongate-obovoidal shaped) มี 2 เซลล์ ใส conidiophores ตรงยาว แตกกิ่งก้าน พบ denticles หรือ conidial head บริเวณส่วนปลาย รองลงมาคือ *Monacrosporium* spp. พบร้อยละ 10 conidia รูปร่างคล้ายลูกรักบี้ (spindle-shaped) มี 2-5 ผนังกั้น ส่วนปลายมี truncate base ส่วน Conidiophores ตั้งตรง บางชนิดแตกกิ่งก้าน และ *Paecilomyces* spp. พบร้อยละ 5 conidia ขนาดเล็กต่อเป็นโซ่ ลักษณะกลม หรือหัวท้ายแหลม (ellipsoidal to fusiform) 1 เซลล์ ส่วนปลาย Conidiophores มีลักษณะ synnemata และ phialides สำหรับไอโซเลทที่ยังไม่สามารถจำแนกได้ พบร้อยละ 3 ดังแสดงในภาพที่ 1-4

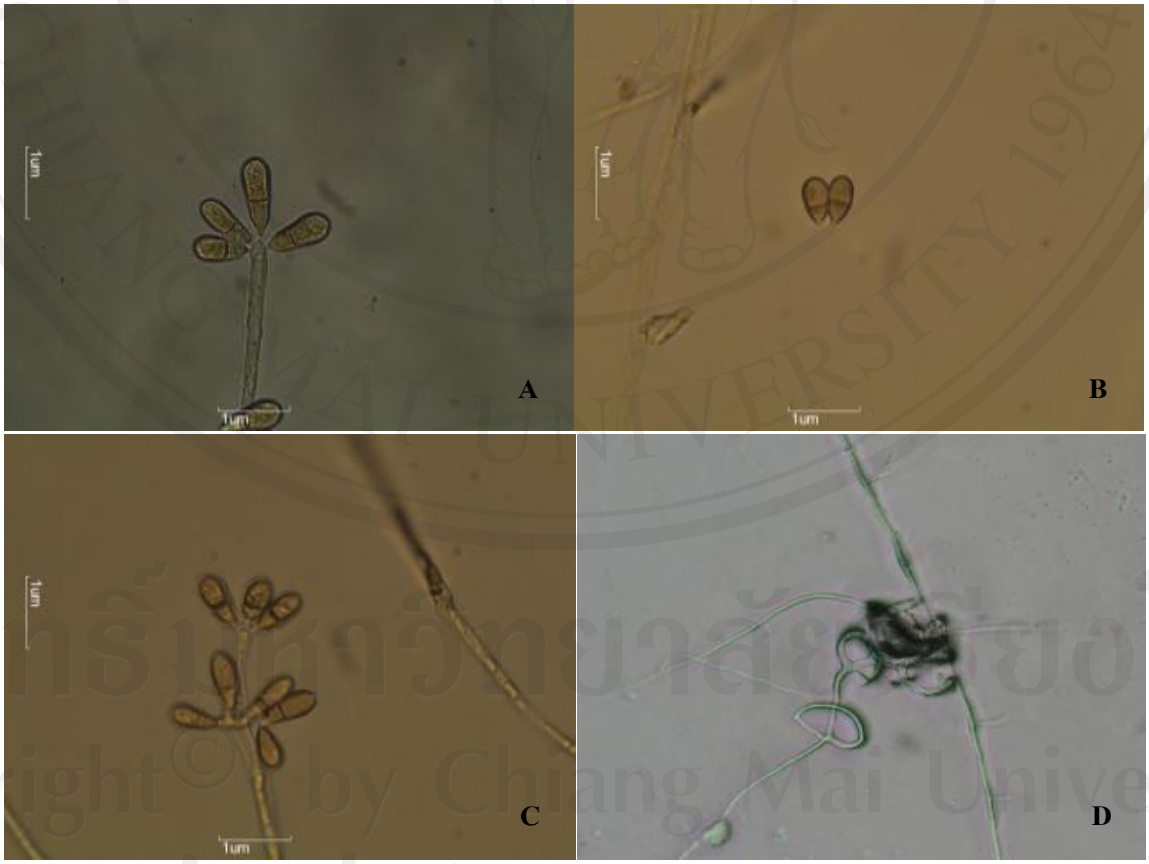

FIG. 1. A-C. Obovoidal to ellipsoidal conidia and conidiophores D. Trapping structures of *Arthrobotrys* spp.

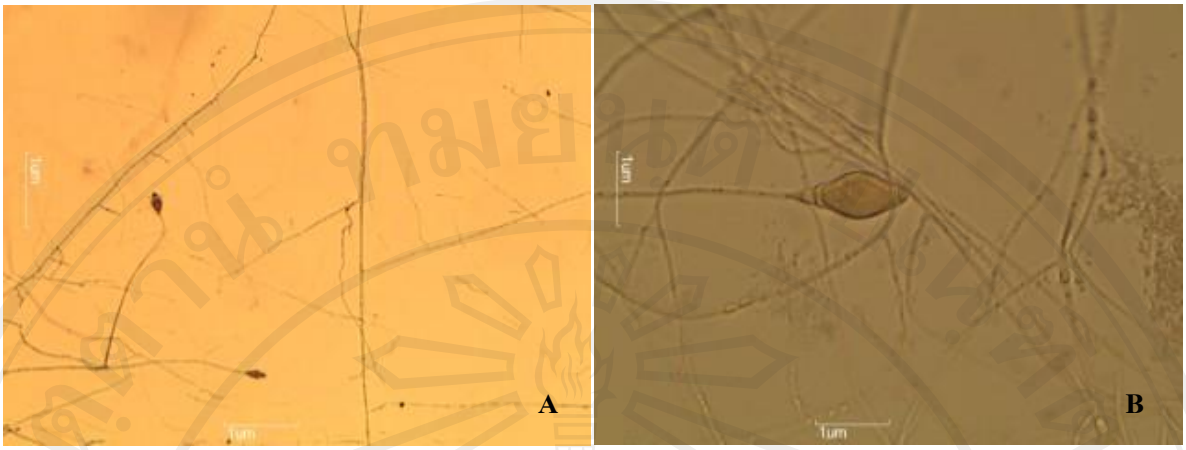

FIG. 2. A-B. Spindle-shaped conidia and conidiophores of *Monacrosporium* spp.

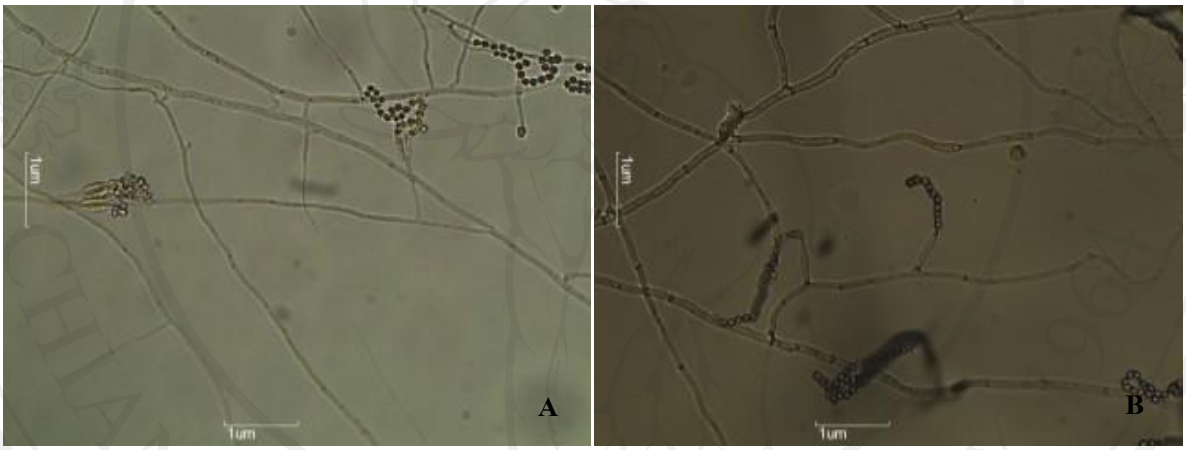

FIG. 3. A-B. Globose or ellipsoidal to fusiform conidia and conidiophores of *Paecilomyces* spp.

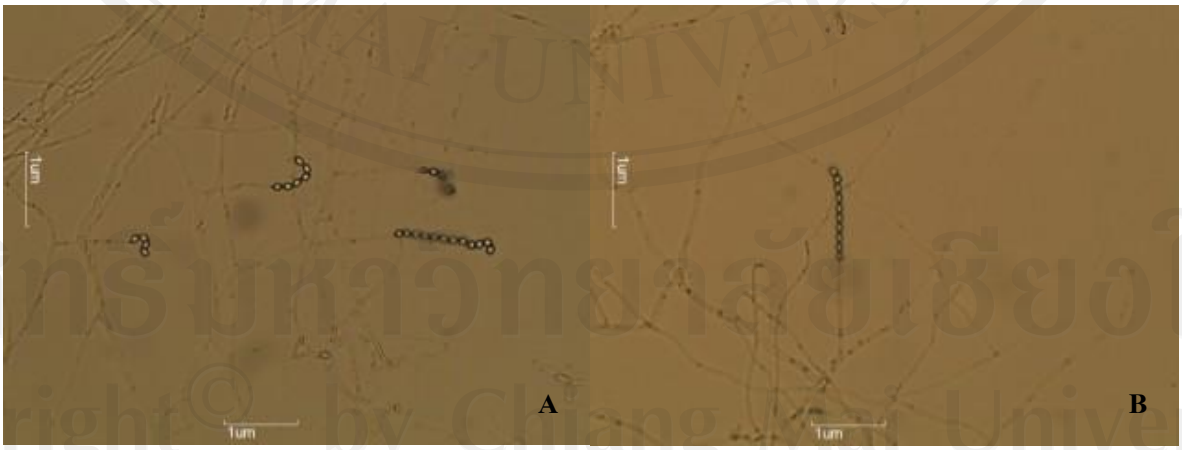

FIG. 4. A-B. Ellipsoidal conidia and conidiophores of MPl 007

2. การทดสอบประสิทธิภาพการทำลาย J2 และไข่ ในสภาพห้องปฏิบัติการ

ผลการตรวจสอบพบว่า เชื้อราปฏิปักษ์ จำนวน 8 ไอโซเลท สามารถทำลาย J2 และไข่ ตั้งแต่ 3 วัน หลังทดสอบ โดยไอโซเลท DLo 001 สามารถดักจับ J2 ภายใน 3 วัน มากที่สุด 24 % รองลงมาคือ MTo 010 (15%) นอกจากนี้ยังพบว่า ไอโซเลท DLo 001, MTo 010 และ JDl 001 สามารถดักจับ J2 ได้ 100 % ภายใน 7 วัน (Table 1) สำหรับการทำลายไข่ พบว่า ไอโซเลท WJ 003 และ MPl 007 มีประสิทธิภาพดีที่สุด 75 % และ70% ตามลำดับ

Table 1 Percentage capturing of second-stage juveniles of *Meloidogyne incognita* by eight isolates of nematophagous fungi on Water Agar medium

Area (Village, District, Amphoe, Province)	**Code-isolate**	**% capturing of J2 of M. *incognita*[1]**		
		3 day	**5 day**	**7 day**
Dong Leo Sri, Ban Hong, Ban Hong, Lumpoon	DLo 001	24	85	100
Mae Tho, laolee, Hot, Chiang Mai	MTo 010	15	86	100
Mae Sariang, Aom pai, Hot, Mae Hong Son (2)	MSr 018	0	75	90
Jadeemaekuey, Maejadeemai, Sansai, Chiang Mai	JDl 001	9	83	100
Mae Phae, Sa Meang Noa, Sa Maeng, Chiang Mai	MPa 007	0	76	90
Mae Sariang, Aom pai, Hot, Mae Hong Son (1)	MSr 007	0	70	91
Mae Pun Luang, Wiang, Wiang Pa Pao, Chiang Rai	MPl 007	0	0	0
Ban Jan, Wat Jun, Mae Jam, Chiang Mai	WJ 003	0	0	0

[1] Average of 3 replications per each isolates

3. การบันทึกข้อมูลพื้นฐานหรือลักษณะเฉพาะเชื้อราประสิทธิภาพสูงที่ถูกคัดเลือก

ผลการจำแนกลักษณะทางสัณฐานวิทยาเชื้อราปฏิปักษ์แต่ละไอโซเลท ได้แก่ ไอโซเลท DLo 001 และ MTo 010 คือ *Arthrobotrys oligospora* MSr 018 คือ *Arthrobotrys conoides* MSr 007 คือ *Arthrobotrys musiformis* JDl 001 และ MPa 007 คือ *Monacrosporium thaumasium* WJ 003 คือ *Paecilomyces lilacinus* ในขณะที่MPl 007 ยังไม่สามารถจำแนกชนิดได้ สำหรับรูปแบบการทำลาย J2

พบว่า เชื้อรา *Arthrobotrys* spp. 4 ไอโซเลท สร้างตาข่ายแบบ three-dimensional network หรือ Adhesive nets รัดตัวไส้เดือนฝอย ซึ่งขนาดหรือจำนวนตาข่ายจะแตกต่างกันในแต่ละไอโซเลท เช่นเดียวกับ *Monacrosporium* spp. ทั้ง 2 ไอโซเลท ส่วน *Paecilomyces lilacinus* กำจัดไข่ด้วยการสร้างเส้นใยแทงทะลุผนังไข่ ลักษณะคล้ายกับไอโซเลท MPl 007 ตามรายงานของ Ahren and Tunlid (2003) นอกจากนี้ยังพบว่า เชื้อรา *Arthrobotrys musiformis* ไอโซเลท MSr 007 ที่เลี้ยงบนอาหารแข็ง เช่น WA PDA CMA PCA สามารถผลิตกลิ่นคล้ายแอมโมเนียด้วย รายละเอียดแสดงใน (Table 2)

Table 2 Characterization of eight isolates of nematophagous fungi on Water Agar medium 7 day

Species	Code-isolate	Characterization of Nematophagous fungi		
		Trapping structures	Volatile	Number[1] of traps per disc
Arthrobotrys oligospora	DLo 001	Three-dimensional network	-	7
Arthrobotrys oligospora	MTo 010	Three-dimensional network	-	9
Arthrobotrys conoides	MSr 018	Adhesive nets	-	20
Monacrosporium thaumasium	JDl 001	Three-dimensional network	-	5
Monacrosporium thaumasium	MPa 007	Three-dimensional network	-	4
Arthrobotrys musiformis	MSr 007	Adhesive nets	Ammonia	3
Unknown	MPl 007	Penetration of hyphae	-	0
Paecilomyces lilacinus	WJ 003	Penetration of hyphae	-	0

[1]Average of 3 replications per each isolates

สรุปผลการทดลอง

สามารถคัดเลือกเชื้อราปฏิปักษ์ไส้เดือนฝอยรากปม M. *incognita* ประสิทธิภาพการทำลายสูงมากกว่า 70 เปอร์เซ็นต์ จากแปลงปลูกพืชผักบนพื้นที่สูง 8 ไอโซเลท แบ่งเป็น (1) ชนิดที่ทำลาย J2 จำนวน 6 ไอโซเลท ได้แก่ เชื้อรา *Arthrobotrys* spp. 4 ไอโซเลท และ *Monacrosporium* spp. 2 ไอโซเลท

(2) ชนิดที่ทำลายไข่ มี 2 ไอโซเลท คือ *Paecilomyces* spp. และ Unknown โดยเชื้อราแต่ละชนิดมีรูปแบบการกำจัดไส้เดือนฝอยแตกต่างกันในลักษณะ จำนวนและโครงสร้างดักจับไส้เดือนฝอย ความเร็วของการทำลาย รวมทั้งลักษณะ และปริมาณสปอร์ที่สร้างหลังจากเสร็จสิ้นกระบวนการกำจัดไส้เดือนฝอยแล้ว ซึ่งมีส่วนเกี่ยวข้องกับการแพร่พันธุ์หรือการคงอยู่ในสภาพแวดล้อมที่มีการแปรปรวน ดังนั้นการวิจัยและพัฒนาในระยะต่อไป จึงสรุปได้ดังนี้ (1) ทดสอบความสามารถในการปรับตัวเพื่อให้มีชีวิตของเชื้อราสภาพต่างๆ เช่น ความเป็นกรดด่าง อุณหภูมิ แสง สารพิษ (2) ทดสอบอาหารเลี้ยงเชื้อเพื่อเพิ่มการเจริญเติบโตและปริมาณสปอร์ (3) ทดสอบประสิทธิภาพการควบคุมไส้เดือนฝอยรากปมในแปลงปลูกพืช และ (4) พัฒนาเป็นชีวภัณฑ์รูปแบบต่างๆ ที่เหมาะสมกับการใช้งานของเกษตรกร

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O-5

Influence of Fungicide and Herbicide to Nematophagous Fungi Against Root-knot Nematode

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The purpose of this research was to study effect of various pesticides. Five popular fungicides and three herbicides were tested under *in vitro* conditions on the growth and sporulation of 8 isolates of efficient nematophagous fungi against root-knot nematode which divided in 3 genera, *Arthrobotrys* spp., *Monacrosporium* spp. and *Paecilomyces* spp. After 10 days incubation, metalaxyl mixed mancozep 68 W/G (1,700 ppm a.i.) caused almost complete inhibition of all fungal radial mycelial growth, with significantly (P=0.05), at all tested concentrations consisting of recommended rate, double rate, half of recommended rate and one-third of recommended rate in comparison to non treated dishes. Fosetyl aluminium 80 WG (8,000 ppm a.i.) caused 100% inhibition of all fungal radial mycelial growth at double rate and recommended rate as well as half of recommended rate inhibiting 6 isolates of nematophagous fungi, except 2 isolates of *Paecilomyces* genera, at 100%. Quintozene mixed etridiazole 30% W/V EC containing 900 ppm a.i. caused 100% inhibition of all fungi at double rate and 100% inhibition at recommended rate in 6 isolates of the fungi, similarly result of fosetyl aluminium at half of recommended rate. In addition, propamocarb hydrochloride 72.2 % W/V SL containing 722 ppm a.i. has a slightest tendency affectation of the fungal radial growth and sporulation. Radial mycelia of *Arthrobotrys* spp. and *Monacrosporium* spp. have highest sensibility derive from paraquat dichloride 27.6 %W/V SL (1,725 ppm a.i.) so theirs mycelia were inhibited at 100%. Oxyfluorfen 23.5 % W/V EC was second affecting herbicide that involving a quantity of sporulation. However, all pesticides have not an impact on morphological character of colony surfaces such as color and texture including spore appearance and their germination. This result indicated that soil pesticides are toxic to nematophagous fungi; therefore, theirs capacity may be decrease.

Key words: nematophagous fungi, root-knot nematode, fungicide, herbicide

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Selection of efficient nematophagous fungi against root-knot nematodes in the highland cultivated area

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A number of highly virulent nematophagous fungi were recovered by soil sprinkling technique from both infested nematode plantation sand areas rich in organic matter in four provinces of Thailand. Four isolates of genus *Arthrobotrys* and two isolates of genus *Monacrosporium* selected from forty-five soil samplings damaged 90-100% second stage juveniles (J2) of root-knot nematodes (*Meloidogyne incognita*) using adhesive nets structures while two isolates, genus *Paecilomyces* and genus *Pochonia* infected 70-75% of eggs by means of appressoria. Results of morphological and molecular identification were generally concordant. The morphological and molecular data were in agreement for four fungal isolates, DLO1-001 (*Arthrobotrys oligospora*), MTI2-001 (*A. oligospora*), API3-001 (*Arthrobotrys conoides*) and MSO1-001 (*Arthrobotrys musiformis*). The conidiophore patterns and conidia classified JDI1-001 and MPI1-003 as genus *Monacrosporium*. Nevertheless, the 5.8s-ITS2-28s rDNA sequence data using ITS1 and ITS4 primers aligned them with *Arthrobotrys thaumasia*.

Key words: nematophagous fungi, biological control, highland, root-knot nematodes, *Meloidogyne incognita*, ITS

Introduction

Root-knot nematodes *(Meloidogyne* spp.) affected plants showing symptoms of stunting, wilting or yellowing including lumps or galls on roots. Control strategies for root-knot nematodes should be based on density reduction in soil through sustainable and eco-friendly approaches. Nematophagous fungi are natural enemies of nematodes and around 160 species are known in this carnivorous group (Wikipedia, 2012d). They are found in most fungal taxa: Ascomycetes and their hyphomycete anamorphs, Basidiomycetes,

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Zygomycetes, Chytridiomycetes and Oomycetes (78 Steps Health Journal, 2012). These fungi can be classified into four major groups according to their infective strategies: trapping, endoparasitic, opportunistic and toxic fungi (Xue-Mei and Zhang, 2011). They use special mycelial structures to penetrate the nematode cuticle, invade and digest nematodes (Nordbring-Hertz *et al*., 2006). Accordingly, these fungi became of interest as bio-control agents against plant- and animal-parasitic nematodes. Persmark *et al*. (1996) showed that many nematophagous fungi have been found most frequently in the rhizospheres of plants. Nevertheless, these fungi have more complex relationship with their nematode hosts and ecology, since they also have an ability to live saprophytically (Ming-He, 2006). Morphological characteristics used for species identification of isolates included colony growth and culture characteristics on media, conidiophore branching pattern and arrangement including conidial morphology and quantity. These criteria were generally useful for species identification but in some cases interspecies overlap occurred. Therefore, molecular techniques are essential for confirmation of cultural and morphological species identification. The ITS region is now perhaps the most widely sequenced DNA region used for fungal identification. It has typically been most useful for molecular systematics at the species level, and even within species e.g., to identify geographic races (Vilgalys lab, 2012).

The objectives of this study were to select efficient nematophagous fungi against root-knot nematodes for highland plantation applications and to classify the genera and species of selected fungi based on morphological characteristics and molecular techniques.

Materials and methods

Collection of soil samplings and Isolation of nematophagous fungi

Randomized samples of 500 g of soil were collected from rhizospheres from infected root-knot nematode plantations and adjacent areas rich in organic material in highland areas in Thailand. Approximately 1 g of each soil sampling was sprinkled on the surface of three water agar (WA) Petri dishes containing antibiotics (0.05% streptomyc in sulphate and 0.01% chloramphenicol) together with a suspension of root-knot nematode eggs added as bait. The Petri dishes were incubated at room temperature (25-30ºC) for 3 and 5 days and then examined by microscope at low magnification for the appearance of trapped nematodes, trapping organs and conidia. Pure cultures of the fungi were made by single spore isolation technique.

***In vitro* predacity of nematophagous fungi against *Meloidogyne* spp.**

Cultures of each fungus were grown in a 1:10 corn meal agar (CMA) medium containing antibiotics. Second stage juveniles (J2) or egg sacs were isolated from lettuce root galls and washed 5 times with sterilized distilled water. Two drops of water containing 100 J2 or eggs of *Meloidogyne incognita* was immediately inoculated into each Petri dish. Three Petri dishes served as replicates. They were kept at 25°C and the observations on trapping structures and trapped nematodes were taken at 3, 5 and 7 days under a microscope at 100×. Verification of the formation of predaceous structures and capturing of nematodes were recorded and percentages calculated.

Identification of nematophagous fungi based on morphological characteristics

The competent fungal cultures were maintained on CMA at 27ºC for 10 days; to observe morphological characteristics and slide-cultures were incubated for a week after inoculation of the fungi. The isolates were analyzed based on conidiophore branching patterns, and arrangement and mode of conidia production using the online database programs Mycobank, Index Fungorum and keys to the nematode-trapping genera of hyphomycetes and some similar genera developed by Annemarthe (no date)**.**

Identification of nematophagous fungi based on molecular techniques

DNA extraction

Each isolate of selected fungi was grown on PDA at room temperature. The mycelia were ground in liquid nitrogen with mortar and pestle to a fine power. The genomic DNA was extracted using the DNA Trap I (DNA TEC Cat NO.100-1009) according to the manufacturer's instructions. Powdered mycelium was suspended in detergent solution (700 µl of extraction buffer), incubated at 65 ℃ for 60 min and put on ice box for 5 min. Extraction was with 120 µl neutralizer, mixed and put on ice box for 10 min. The cellular debris was pelleted by centrifugation for 5 min at 10,000 rpm. 1.5 ml of supernatant was mixed with 500 µl of trapping buffer and left at room temperature for 10 min. The mixture was centrifuged at 10,000 rpm for 1 min to harvest any pellets after discarding the supernatant. Samples were washed with 50 µl of washing buffer I and centrifuged at 10,000 rpm for 1 min to harvest whole pellets. The previous step was repeated with washing buffer II. The pellets were dried in an incubator at 65 °C and 10 µl of elution buffer was added before centrifugation.

The mixture was incubated at 65 °C for 30 min and centrifuged at 10,000 rpm for 5 min. The supernatant with DNA was kept at -20 °C prior to final characterization.

Determination of DNA concentration

DNA quality and quantity were determined by comparing with standard DNA intensity using an agarose gel electrophoresis protocol. A standard 1% (w/v) agarose gel prepared in Tris-Acetate-EDTA (TAE) electrophoresis buffer was used for analysis of total DNA preparations from fungal isolates and PCR amplicons. One gram of agarose powder was dissolved in 100 ml of 1X TAE buffer and microwaved for 2 min. The gel was cast with a sample slot comb. After approximately 30 min, the gel had solidified sufficiently to allow comb removal. TAE buffer was added in electrophoresis tank after submerging the gel. The samples in 6X gel-loading buffer were loaded into individual gel slots and run at 100 volts for 30 min. The gel was stained with ethidium bromide solution (10 µl /100 ml of buffer) for 10 min before viewing and photographing using a long wave UV transilluminator.

PCR amplification of the ITS region

PCR reaction and digestion of amplified fragments were performed according to the procedures of Korabecna, 2007; Esteve-Zarzoso, 1999. The 5.8s-ITS2-28s rDNA gene was amplified by PCR using the internal transcribed spacer primers ITS1 (5´TCC GTA GGT GAA CCT GCG G 3´) and ITS4 (5´TCC TCC GCT TAT TGA TAT GC 3´) (White, 1990). The PCR amplification was carried out using the GeneAmp^{R} PCR System 9700 (Applied Biosystems). Twenty µl of reaction mixture contained 10 ng of template DNA, 0.25 µM of each ITS1and ITS4 primer, PCR buffer which was comprised of 100 mMTris-HCL (pH 9.0), 500 mMKCl, 2.0 mM $MgCl_2$), 200 µM dNTPs and 0.6 unit of *Taq* DNA polymerase. The PCR amplification was programmed to carry out an initial denaturation step at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and elongation at 72 °C for 7 min, followed by 1% (w/v) agarose gel electrophoresis and purification with PCR kit.

Sequencing and phylogenetic analysis

Sequences of PCR products were obtained from both strands with ITS1 and ITS4 primers using the dideoxy chain termination method. The PCR products generation was carried out with the BigDye® Terminator v3.1 cycle

sequencing kit, (1st BASE, Singapore) and automated DNA sequencer following the manufacturer' s instructions. The Sequencher version4.7 software was used to assemble, edit and generate high-quality sequences. Sequence similarity analyses were performed using the Basic Local Alignment Search Tool (BLAST) in GenBank or databases of National Center for Biotechnology Information: NCBI BLAST Assembled RefSeqGenomes program (NCBI, 2012).

The multiple sequence alignment program: MAFFT version 6 and GeneDoc version 2.7 was used to align nucleotide sequences. The phylogenetic tree was obtained from data using one of three equally parsimonious trees obtained through 1,000 replications of an heuristic search with random, stepwise sequence addition by *PAUP* version 4.0b10 (Phylogenetic Analysis Using Parsimony). Additional ITS sequences of nematophgous fungi were retrieved from GenBank.

Results

Collection of soil samplings and Isolation of nematophagous fungi

One hundred and three isolates were obtained from the Chiang Mai area, two from Chiang Rai, four from Nakhonsawan and seven from Mae Hong Son. One hundred and one nematophagous fungi classified as trapping fungi, five isolates as endoparasites and ten as egg parasites were isolated from forty-five soil samplings. The genus *Arthrobotrys* sp. forming either adhesive nets or constricting rings was most commonly found (75%) followed by *Monacrosporium* sp. (12.93%) which formed non-constricting rings or adhesive knobs (stalked knob) structures, egg parasite *Paecilomyces* sp. (7.76%) and endoparsite *Meristacrum* using adhesive spores damaged J2 (4.31%) (Figure 1).

In vitro predacity of nematophagous fungi against *Meloidogyne* spp. was done. Each fungal isolate varied in their capacity to capture and kill nematodes. A few fungal isolates showed quickness in capturing nematodes. Notable, a destructive process was initiated by most isolates after 5 days. Seven fungal isolates, JDI1-001, MTI2-001, MSO1-001, MPI1-003, KJO1-003, WJI1-003 and API3-001 appeared to have high destructive capacities against root-knot nematodes. After 7 days, JDI1-001(*Monacrosporium* sp.) damaged 100% of second stage juveniles (J2) of root-knot nematodes and MTI2-001(*Arthrobotrys* sp.). MSO1-001(*Arthrobotrys* sp.), MPI1-003(*Monacrosporium* sp.) and API3-001 (*Arthrobotrys* sp.) damaged at 91.2%, 90.1% and 90.0%, of J2 nematodes respectively while KJO1-003 (*Pochonia* sp.) and WJI1-003 (*Paecilomyces* sp.) attacked 70.1 and 75.1% of eggs, respectively (Figure 2).

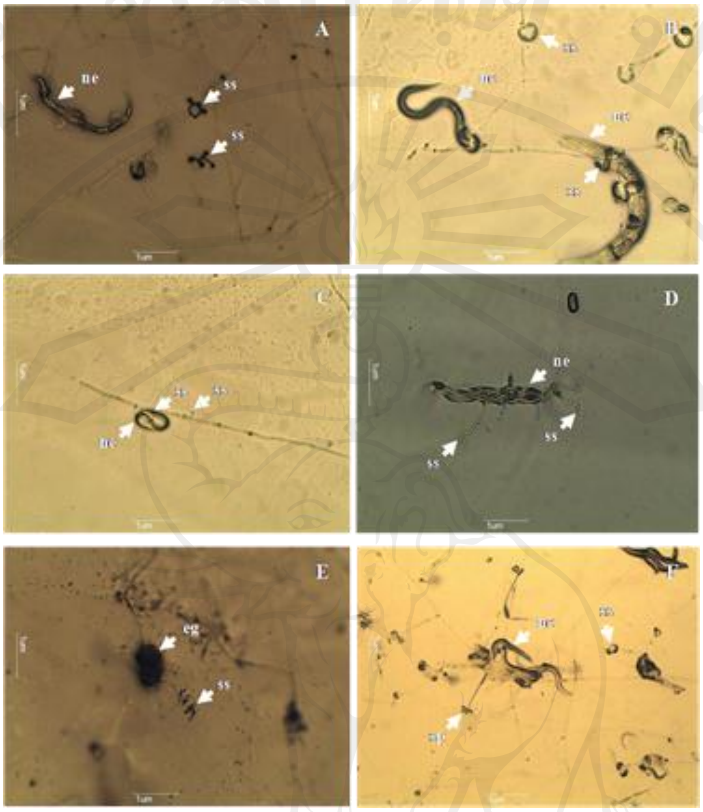


Fig. 1. Characteristics of captured nematodes(ne) or egg (eg) by special structures (ss) of some nemathophagous fungi; A. adhesive nets B. constricting rings C. adhesive knobs D. adhesive spores E. hyphal tips F. sporulation (sp)

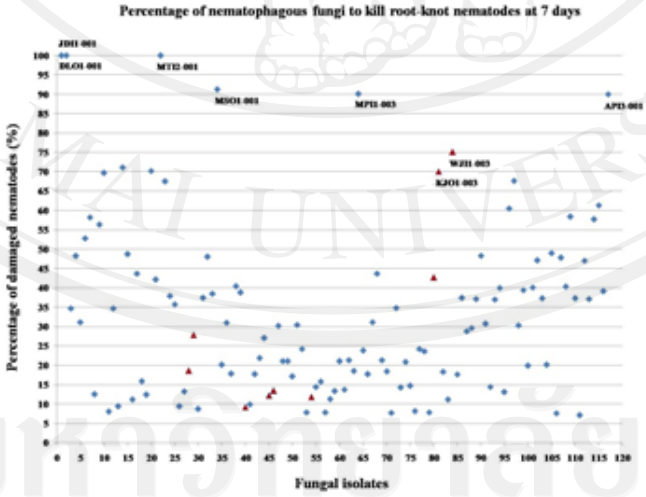


Fig. 2. Percentage of nematophagous fungi capable of killing root-knot nematodes at 7 days
● Damaging nematodes; ▲ Attacking egg of nematode

Identification of nematophagous fungi based on morphological characteristics

DLO1-001 and MTI2-001 were classified as *Arthrobotrys oligospora*. Colony textures on corn meal agar (CMA) of these fungi were fuzzy and powdery, respectively, with dirty white surface color, but the reverse side of the colonies was colorless. Mycelia grew shallowly in light concentric zones and produced a wooly pattern. Conidiophores were simple and erect, ranged in length from 200-450µm, proliferated repeatedly and sporulated heavily. Conidia were obovoidal to pyriform. Submedially, 1-2 septa were observed which sometimes showed the site of slight constrictions. The conidia of DLO1-001 were 33.10±1.41 µm long ×12.90±0.85 µm wide while conidia of MTI2-001 were 29.10±1.55 µm long×12.25±0.85 µm wide.

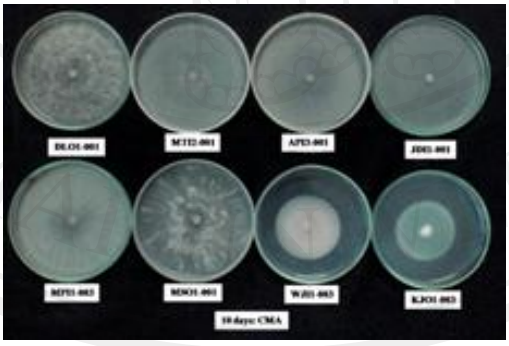
API3-001 was grouped in *Arthrobotrys conoides*. Colony texture was powdery with a dirty white color. In addition, API3-001 produced a colorless mycelial substrate and thin aerial mycelia. Conidiophores were erect, rarely branched, up to 400 µm in length, and proliferated repeatedly causing heavy conidial production. The conidiogenous heads were irregularly swollen, sometimes elongate and had short denticles. Conidia were elongate-obovoidal, with one median septum and slight constriction and measured 38.10±1.07 µm in length ×12.90±1.07 µm in width.

Monacrosporium thaumasium was the designation of JDI1-001 and MPI1-003. The surface and reverse colony color of these fungi were white and colorless, respectively. Colony textures were powdery, but differences in zonation were noted; JDI1-001 had a wooly appearance and MPI1-003 had a slight radial furrowing. Most conidiophores were simple, 150-300 µm in length, had 1-2 small perpendicular branches, and consequently these fungi produced a moderate number of conidia. Spindle-*shaped* conidia were detected on media. At their widest part conidia measured 23.15±1.09 µm and most often had two, equidistant septa.

MSO1-001 was classified as *Arthrobotrys musiformis*. Colony texture of this fungus was fuzzy and dirty white in color, but had limited growth in the center. Furthermore, the mycelial substrate was colorless and thin. Microscopically and by measurement the fungus was most similar to the genus *Arthobotrys* in that it had erect conidiophores, averaging 272.50±54.95 µm in length. However, this isolate rarely produced side branchs and proliferated subapically to produce a candelabrum-like branching system, each branch bearing a single terminal conidium. Conidia were elongate-obovoidal to ellipsoidal and 1-septate slightly below the middle. Conidia averaged 30.85±1.35 µm long×13.05±0.94 µm wide.

WJI1-003 was categorized as *Paecilomyces lilacinus*. Colonies were relatively slow-growing. Colony surface texture was velvety with a light concentric pattern consisting of numerous conidiophores and heavy sporulation. Aerial mycelium was at first white and changed to shades of light purple or sometimes was uncolored. Conidiophores were 30.25±7.34 µm in length, occasionally forming 2-4 layers of loose synnemata which had stalks with roughened thick walls. Verticillate branches with whorls of 2 - 4 phialides were often abundant. Phialides were 26-30 ±6-8 µmin length, consisting of a swollen basal portion tapering into a short distinct neck. Conidia in divergent chains were ellipsoidal to fusiform. They were smooth-walled to slightly roughened, hyaline, but purple in mass. Conidia were 3.125±0.22 µm long×3.05±0.15µm wide.

KJO1-003 was identified as *Pochonia chlamydosporia.* A creamish white and slight cottony colony was observed on CMA. Colony texture was wooly. Its aerial mycelium had shallow growth and a thin form. Conidiophores were usually prostrate and little differentiated from the vegetative hyphae, but sometimes erect and differentiated. Conidiogenous cells were phialides, tapered to a narrow tip, and were hardly visible and solitary. Conidia were transversely positioned on phialides and formed in small slimy heads. Phialides originated from prostrate hyphae, were solitary and up to five per node. Conidia were subglobose, ellipsoidal to rod-shaped, isodiametric-polyhedric, or falcate with blunt ends, 3-3.5 µm wide and mostly adhered on globose heads or chains. Colony characterization of eight nematophagous fungi are shown in Figure 3 and Table 1.



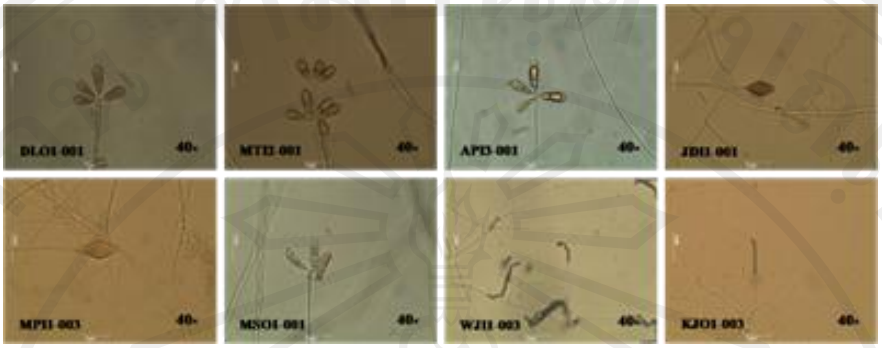


Fig. 3. Characteristics of eight fungal in colony textures, conidiophore patterns and conidia.

Table 1. Colony characterization of eight nematophagous fungi

Isolate	Genera and Species	Colonydiam $^{1/}$ 5 days (cm)	Colony character			Zonation	Sporulation
			Texture	Surface color	Reverse color		
DLO1-001	*Arthrobotrys oligospora*	7.5-8.0	Fuzzy	Dirty white	colorless	Light concentric zones	Heavy
MTI2-001	*Arthrobotrys oligospora*	7.5-8.0	Powdery	Dirty white	colorless	Wooly	Heavy
API3-001	*Arthrobotrys conoides*	7.5-8.0	Powdery	Dirty white	colorless	Wooly	Heavy
JDI1-001	*Monacrosporium thaumasium*	6.5-7.0	Powdery	White	colorless	Wooly	Moderate
MPI1-003	*Monacrosporium thaumasium*	6.5-7.0	Powdery	White	colorless	Slightly radially furrowed	Moderate
MSO1-001	*Arthrobotrys musiformis*	7.5-8.0	Central fuzzy	Dirty white	colorless	Slightly radially furrowed	Moderate
WJI1-003	*Paecilomyces lilacinus*	5.5-5.7	Velvety	Light purplewhite	Slightly purple	Light concentric zones	Heavy
KJO1-003	*Pochonia chlamydosporia*	5.0-5.5	Slight cottony	Creamish white	Slightly creamish	Wooly	Moderate

Identification of nematophagous fungi based on molecular techniques

The genus and species of eight isolates of nematophagous fungi were confirmed by molecular techniques. The 5.8s-ITS2-28s rDNA gene was amplified using the internal transcribed spacer primers: ITS1 and ITS4. The PCR amplified region and the PCR products ranged from 670-740 bp.

Nucleotide comparisons of these fungi using the GenBank and NCBI databases and the BLASTN 2.2.26 program indicated that DLO1-001 and MTI2-001 were *Arthrobotrys oligospora* (91% and 90% homology, respectively). Blast results identified JDI1-001 as *Arthobotrys thaumasia* with a

maximum score (741 bits). MPI1-003 was identified as either *Monacrosporium thaumasium* (601 bits) or *Arthobotrys thaumasia* (597 bits) at the similar maximum identity. Five hundred and eighty nucleic acid query length of MSO1-001 was significantly aligned (91%) and identified this fungus as *Arthrobotrys musiformis*. API3-001 had highly similar sequences with *Arthrobotrys conoides*. KJO1-003 and WJI1-003 had no significant similarity with any genera or species based on molecular data (Table 2).

Table 2. The blast results of rDNA ITS sequences from nematophagous fungi and their closely

Example code[1/]	ITS Blast result [2/]	Maximum Score[3/]	Identity[3/]	Gap[3/]	Accession# [3/]	Reference[3/]
DLO1-001	*Arthrobotrys oligospora*	802	529/580 (91%)	1/580 (0%)	EU977526	Swe *et al*. (Unpublished)
MTI2-001	*Arthrobotrys oligospora*	817	565/631 (90%)	2/631 (0%)	HQ649929	Macia-Vicente *et al*. (Unpublished)
API3-001	*Arthrobotrys conoides*	817	552/616 (90%)	0/616 (0%)	JN191309	Falbo *et al*. (2011)
JDI1-001	*Arthrobotrys thaumasia*	741	509/564 (90%)	4/564 (1%)	AF106526	Hagedorn & Scholler (1999)
MPI1-003	*Monacrosporium thaumasium*	601	464/535 (87%)	4/535 (1%)	FJ380934	Kuo *et al*. (2009)
MSO1-001	*Arthrobotrys musiformis*	745	497/574 (91%)	2/574 (0%)	U51948	Liou & Tzean (1997)
WJI1-003	Non matched	-	-	-	-	-
KJO1-003	Non matched	-	-	-	-	-

Related sequences in GenBank during June 2012
[1/]Isolated from agricultural soil, Thailand
[2/]Referenced program by Zhang *et al*. (2000)
[3/] Reported first sequences producing significant alignment

Phylogenetic analysis

In this study, ITS sequences of selected fungal isolates were compared with those of 36 published nematophagous fungi; *Arthrobotrys* spp., *Monacrosporium* spp. and *Duddingtonia* sp. retrieved from GenBank. *Neurospora pannonica* and *Sordaria fimicola* were used as the out group.

Multiple sequence alignment was used to infer the maximum likelihood tree. Of the remaining 821 included characters. 244 characters were constant

and 203 variable characters were parsimony-uninformative so the number of parsimony-informative included characters was 374. Nucleotide sequences based on the rDNA ITS region indicated a relationship between genotypes of some fungal isolates. Most of the selected nematophagous fungi were harmoniously clustered as blast groups. The phylogenetic relationships of Orbiliaceae which include *Arthrobotrys oligospora* (DLO1-001 and MTI2-001), *A. conoides* (API3-001) and *A. musiformis* (MSO1-001) were well defined. While the phylogenetic relationships of JDI1-001 and MPI1-003 were not clear, they had 99% BSV and nearby groups with *A. thaumasia*, *Monacrosporium thaumasium*, *M. microscaphoides*, *A. multisecun* and *M. eudermatum* as shown in Figure 4.

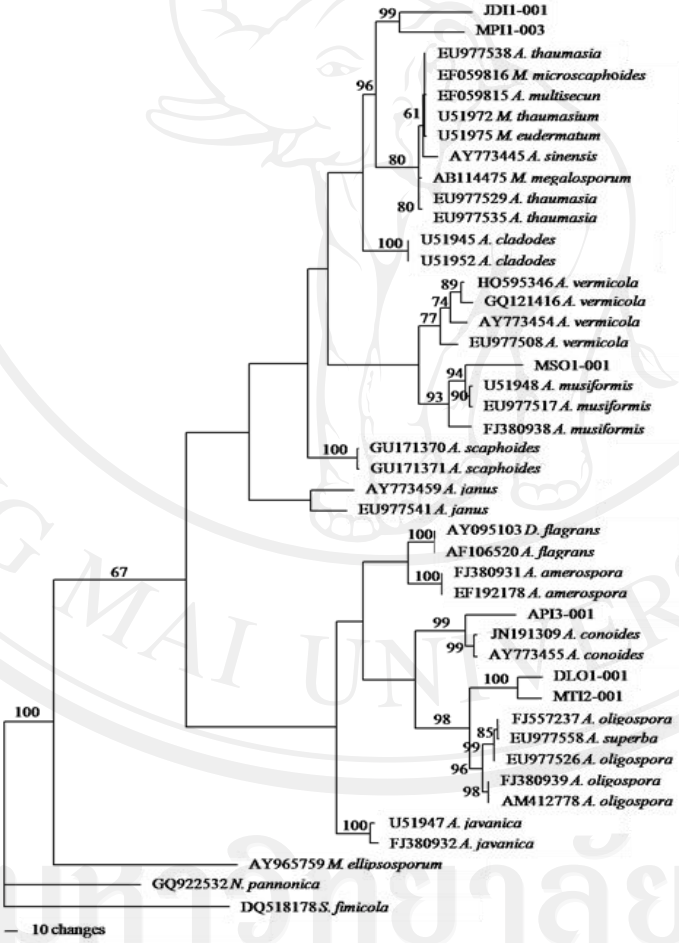


Fig. 4. One of 6 equally most parsimonious trees inferred from a heuristic search of the ITS1-5.8s-ITS2 rDNA sequences alignment of 44 isolates of *Arthrobotrys* and related genera. The size of the branches is indicated with a scale bar. Length=1,525, Cl=0.609 and Rl=0.723

Discussion

Eight isolates in three genera of competent nematophagous fungi which were collected from soil in root-knot nematode-infested areas and areas rich in organic matter from Thailand including *Arthrobotrys* sp., *Monacrosporium* sp., and *Paecilomyces* sp. Their taxonomic classification and infection structures were similar taxonomically and morphologically (infection structures) to those reported by Nordbring-Hertz *et al*. (2006), Gray (2002) and Jersys *et al*. (2009). In *in vitro* predation experiments, some isolates of the collected fungi which are nematode trapping species, endoparasites and egg parasites had low capacities to damage either second stage juveniles (J2) or eggs of root-knot nematodes. The parasitic ability of nematophagous fungi maybe related to a broad range of factors including the level of their saprophytic or absolute parasitic ability (Nordbring-Hertz *et al*., 2006) pH, moisture, organic matter, host suitability (Gray, 2002) nutrient levels, physical habitats, competitive conditions and compounds secreted by the host nematode along with the interactions in biochemical, physiological or morphological responses (Mariam, 2008). Morphological classification of the nematophagous fungi described in this research was based on such characteristics as colony diameter, culture appearance (texture, surface and reverse colouration, zonation) and colony growth rate. It is understood that the specific colony characteristics of each fungus may be different depending on a type of culture medium used as Sharma and Pandey (2010) reported Morphological identification and nucleotide comparisons at the 5.8s-ITS2-28s rDNA gene using ITS1 and ITS4 primers were in agreement for four fungal isolates of genus *Arthobotrys*. Blast results identified JDI1-001 as *Arthobotrys thaumasia*; nevertheless, conidiophore patterns and conidia classified JDI1-001 and MPI1-003 as *Monacrosporium thaumasium*. However, Index Fungorum (2012) reported that *M. thaumasium* and *A. thaumasia* (Drechsler) S. Schenck, W.B. Kendr. & Pramer, were actually synonymous *Can.* J. Bot. 55(8): 984 (1977). WJI1-003 and KJO1-003 were morphologically categorized as *Paecilomyces lilacinus* and *Pochonia chlamydosporia*, but could not be classified molecularly based on nucleotide blast format. This result may be based on the unsuitability of the sequence region and primer selection, and/or the PCR protocols used following the research of Peter and Myrian (2006) and Ciancio *et al*. (2005). The genera *Paecilomyces* and *Pochonia* include a few species that damage nematodes and they had distinct colony characteristics on most growing media so molecular identification may not be necessary.

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Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi

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Sumalee Mensin, Kasem Soytong, Robert J McGovern and Chaiwat To-anun (2013). Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi. Journal of Agricultural Technology 9(4):953-961.

The soil application of nematophagous fungi represents a potentially useful component for the sustainable management of plant parasitic nematodes. Little is currently known about the effect of agrochemicals on these fungi. Fourteen commonly used agricultural pesticides (insecticides, fungicides and herbicides) were incorporated into Potato Dextrose Agar at 1/3x, 1/2x, 1x and 2x the recommended rate to determine their *in vitro* effect on eight isolates of nematophagous fungi; *Arthrobotrys oligospora* (DLO1-001), *Arthrobotrys oligospora* (MTI2-001), *Arthrobotrys conoides* (API3-001), *Arthrobotrys thaumasium* (JDI1-001), *Arthrobotrys thaumasium* (MPI1-003), *Arthrobotrys musiformis* (MSO1-001), *Pochonia* sp. *(*KJO1-003) and *Paecilomyces* sp. (WJI1-003). The isolates were indigenous to Thailand and were parasitic to root-knot nematodes. All insecticides at all rates affected the development of all fungi to some extent. The insecticides dazomet, carbaryl and chlorpyrifos, the fungicides metalaxyl mixed with mancozeb, fosetyl aluminium and quintozene mixed with etridiazole, and the herbicides paraquat dichloride and oxyfluorfen caused high mycelial growth and sporulation inhibition. The insecticides lambda-cyhalothrin, dinitrotefuran and methomyl, the fungicides toclofos methyl and propamocarb hydrochloride and the herbicide glyphosate-isopropylammonium were less inhibitory to the fungi examined. *Paecilomyces* sp. and *Pochonia* sp. appeared to be less sensitive to the pesticides tested than *Arthrobotrys* species.

Key words: nematophagous fungi, biological control, root-knot nematodes, *Meloidogyne incognita*, pesticides, pesticide sensitivity, abiotic factors

Introduction

Nematophagous or trapping fungi are microfungi that can capture, kill and digest nematodes (Nordbring-Hertz *et al*., 2006). They represent a

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potentially effective alternative to chemical nematicides for sustainable farming systems (Martin, 2003). Abiotic factors are non-living chemical or physical factor in the environment and ecology. They are known as "density independent factors" (Biology online, 2008b). The capacity of a soil ecosystem to prevent or reduce the spread of a pathogen, parasite, or other deleterious agent in soils is called antagonistic potential. It includes disease suppressiveness, fungistasis, antiphytopathogenic potential and biological buffering. In addition, management of antagonists in the soil requires an understanding not only of the intricate interrelationships between host-parasite and parasite-antagonist, but also of the interactions among these relationships, crop production practices and abiotic factors (Richard, 1992). The interaction of rhizosphere microorganisms and their physiological factors influence fungal growth and sporulation. Each organism generally reacts in different ways related to their survival and establishment characteristics.

Conventional agricultural pesticides may have long-term toxicity that can affect beneficial soil microorganisms including nematophagous fungi especially their growth and sporulation. Even though research on utility of nematophagous fungi as biological control agents has occurred worldwide for decades, data on the potential negative effects of agricultural pesticides on these fungi is extremely rare. Jacobs *et al*. (2003) found that two other fungicides, fenpiclonil and tolclofos methyl, slowed or partially inhibited the growth of *Paecilomyces lilacinus*, *Plectosphaerella cucumerina* and *Pochonia chlamydosporia, in vitro*. Kerry *et al*. (2009) reported *P. chlamydosporia* and *P. lilacinus* showed different levels of sensitivity to fungicides; mancozeb+propamocarb hydrochloride, imazail+pencycuron and azoxystrobin but were tolerant to herbicides (bentazone, pendimethalin and metribuzin). *P. chlamydosporia* was more tolerant to high concentrations of fungicides than *P. lilacinus* in liquid culture.

Root-knot nematodes *(Meloidogyne* spp.) severely affect plant root systems through gall formation that can lead to stunting, wilting and/or yellowing. It is the most economically important nematode pests in the Pacific (Plant Protection, 2005). Nematophagous fungi may provide an important component in a sustainable approach to manage these important soil-borne pathogens. The objective of this study was to examine the *in vitro* effect of agricultural pesticides (insecticides, fungicides and herbicides) commonly used in vegetable production in Thailand on the growth and sporulation of indigenous nematophagous fungi parasitic to root-knot nematodes.

Materials and methods

The *in vitro* morphological sensitivity of eight nematophagous fungi recovered from Thailand including *Arthrobotrys oligospora* DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003 were assessed on 14 different pesticides. These pesticides with their trade names and recommended rates included (1) six insecticides, dazomet (Basamid-G 98 % GR®, 2,450 ppm a.i.), dinotefuran (Starkle-G 1% GR®, 40 ppm a.i.), lambda-cyhalothrin (Karate 2.5 % W/V CS®, 62.5 ppm a.i.), methomyl (Lannate 40 % SP®, 700 ppm a.i.), carbaryl (Sevin 85 % WP®, 2,975 ppm a.i.) and chlorpyrifos (Lorsban 40 % W/V EC®, 1,500 ppm a.i.) (2) five fungicides, quintozene mixed with etridiazole (Terraclor Super X 30% W/V EC®, 900 ppm a.i.), fosetyl aluminium (Aliette 80 WG®, 8,000 ppm a.i.), metalaxyl-M mixed with mancozeb, (Ridomil Gold MZ 65 WG®, 1,700 ppm a.i.), toclofos methyl (Rizolex 50 % WP®, 1000 ppm a.i.) and propamocarb hydrochloride (Previcur - N 72.2 % W/V SL®, 722 ppm a.i.) and 3) three herbicides, paraquat dichloride (paraquat 27.6 % W/V SL®, 1,725 ppm a.i.), glyphosate-isopropylammonium (Glyphosate 48 % W/V SL®, 3000 ppm a.i.) and oxyfluorfen (Goal 2 E 23.5 % W/V EC®, 587.5 ppm a.i.). Each pesticide was tested at 1/3x, 1/2x, 1x and 2x the recommended rate. A stock solution of each chemical was prepared in sterilized distilled water and appropriate quantities were added under aseptic conditions into 250 ml flasks, containing PDA, to achieve the required final concentrations. The amended media were poured into 9-cm-diameter sterilized Petri dishes, under aseptic conditions and allowed to cool. Petri dishes containing non-amended medium served as the control. A fungal culture agar plug (5-mm-diameter) from the colony edge of each fungal isolate was placed in the middle of the Petri dishes. Four Petri dishes of each isolate of each treatment were used as replicates. The inoculated Petri dishes were incubated at room temperature (27±3ºC). Diameters of the resulting colonies were measured at 3, 5, 7 and 10 days. The percentage fungal growth inhibition was calculated and analyzed for statistical comparison. To determine sporulation, five colonized fungal agar plugs (0.4-cm-diameter) were removed from each plate after 10 days incubation and the sporulation assessment methods of Liu & Chen (2002) were followed. Values of sporulation were transformed to log (base 10) to improve homogeneity of variance before being subjected to analysis of variance (ANOVA). Original values of colony diameter were used in variance (ANOVA) "Factorial in Completely Randomized Design". Duncan's New Multiple Range Test (DMRT) Test was used for comparison of means of each treatment.

Results

Insecticide, rate, fungal isolate and their interactions significantly (*P<0.01*) affected fungal growth and reproduction. All insecticides at all rates affected the development of all fungi. All rates of dazomet , carbaryl and chlorpyrifos caused high mycelial growth inhibition (Fig. 1). Growth of most fungi except *Paecilomyces* sp. isolate WJII-003 was inhibited 100% by dazomet at all rates; however, *Paecilomyces* sp. isolate WJI1-003, *A. thaumasium* isolate JDI1-001 and *A. musiformis* isolate MSO1-001 were completely inhibited by dinotefuran treatment after 5 days (data not shown). At 10 days after inoculation, all rates of carbaryl caused 60-100% inhibition of all fungal isolates especially *A. conoides* isolate API3-001. Lambda-cyhalothrin and methomyl had a lower effect on growth of all isolates with the exception of *A. thaumasium* isolate MPI1-003 which was highly sensitive to all rates of methomyl.

Sporulation of most fungi was correlated with growth; non production of conidia was detected in many cases such as with all fungi at all rates of dazomet, five fungal isolates except *A. conoides* isolate API3-001, *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 at all rates of dinotefuran, all fungal isolates except *Pochonia* sp. isolate KJO1-003 at all rates of carbaryl and chlorpyrifos (data not shown). On the other hand, the half-strength (1/2x) and one-third strength (1/3x) recommended rate for lambda-cyhalothrin induced *Paecilomyces* sp. (WJI1-003) to produce higher number of conidia than the non-treated control.

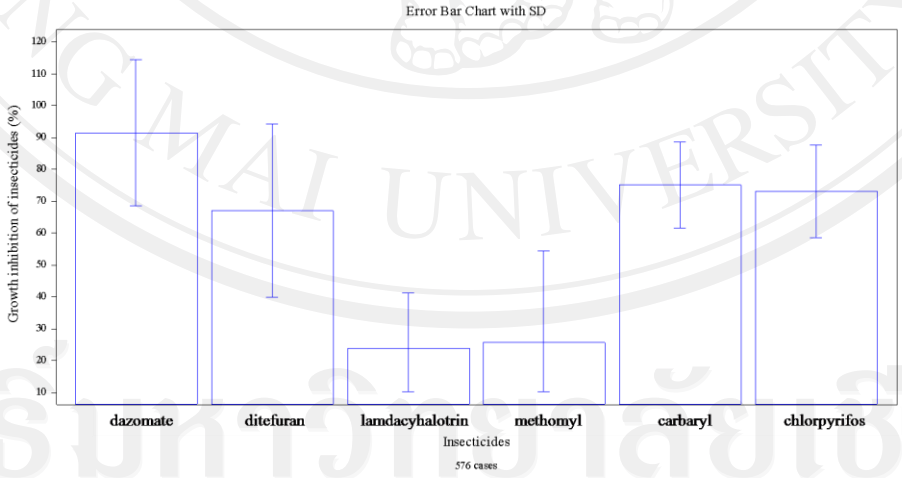

Fig. 1. Effect of various insecticides on growth of nematophagous fungi on Potato Dextrose Agar at 10 days after inoculation. Graph based on the pooled effect of 4 levels (1/3x, 1/2x, 1x, 2x) of the recommended insecticide rate on eight isolates of nematophagous fungi (*P<0.01*).

The analysis of variance using factorial indicated significant effects and interaction of fungal growth inhibition which affected growth and sporulation. These sources were fungicide, usage rate, fungal isolate and all of their interactions.

Metalaxyl mixed with mancozeb caused almost complete inhibition of fungal mycelial growth at all tested concentrations in comparison to the non-treated control (Fig. 2). All rates of fosetyl aluminium caused 100% inhibition at all rates of all fungal isolates except *Paecilomyces* sp. (WJI1-003) and *Pochonia* sp. (KJO1-003). Quintozene mixed with etridiazole (900 ppm a.i.) caused 100% inhibition of most fungi at the double and recommended rates. Only isolate *A. musiformis* (MSO1-001) was significantly inhibited by toclofos methyl; its mycelial growth was inhibited by 65-89% by all rates of the fungicide. Propamocarb hydrochloride had the least effect on the nematophagous fungi; all isolates except *A. conoides* (API1-001) showed little or no sensitivity to the fungicide at 10 days after inoculation. All fungicides at all concentrations affected sporulation by causing partial or complete inhibition and closely paralleled growth inhibition results. Metalaxyl mixed with mancozeb caused non-sporulation of all fungi at all rates as did fosetyl aluminium except for *Paecilomyces* sp. (WJI1-003) (data not shown). Five fungal, *A. oligospora* isolate DLO1-001 and isolate MTI2-001, *A. conoides* (API3-001), *A. thaumasium* isolate JDI1-001 and isolate MPI1-003 did not produce conidia at all rates of quintozene mixed with etridiazole. Propamocarb hydrochloride and toclofos methyl generally decreased sporulation to a lesser extent than the other fungicides.

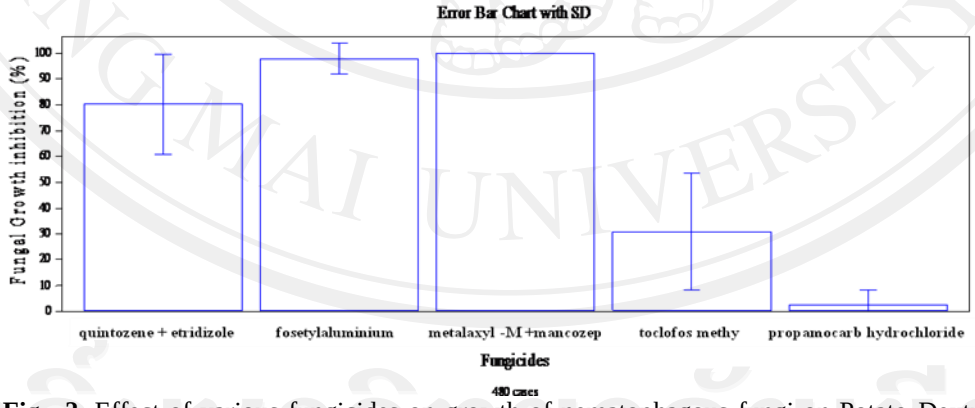

Fig. 2. Effect of various fungicides on growth of nematophagous fungi on Potato Dextrose Agar at 10 days after inoculation. Graph based on the pooled effect of 4 levels (1/3x, 1/2x, 1x, 2x) of the recommended fungicide rate on eight isolates of nematophagous fungi. ($P<0.01$).

Herbicide, usage rate, fungal isolate and all their interactions significantly (*P<0.01*) affected the growth and sporulation at a high probability. Radial mycelia of *A. oligospora* isolate DLO1-001 and isolate MTI2-001, *A. conoides* isolate API3-001, *A. musiformis* isolate MSO1-001, *A. thaumasium* isolate JDI1-001 and isolate MPI1-003 had the highest sensitivity to paraquat dichloride (Fig. 3). Their mycelia were inhibited by 100% at the double and recommended rate. Oxyfluorfen exerted the second strongest growth inhibition effect among herbicides involving reduction of mycelial growth. Only the double rate of glyphosate-isopropyl ammonium caused extensive mycelial growth reduction of *Arthrobotrys* spp. Fungal isolates of *Paecilomyces* sp. and *Pochonia* sp. were generally less sensitive to all rates of the three herbicides than the *Arthrobotrys* spp. tested. Non-sporulation of fungi was detected with at least 90% of all treatments except for *Pochonia* sp. isolate KJO1-003, which only showed a decrease of conidial production. In general, all herbicides caused abnormal mycelial morphology that was directly related to their concentration (Fig. 4).

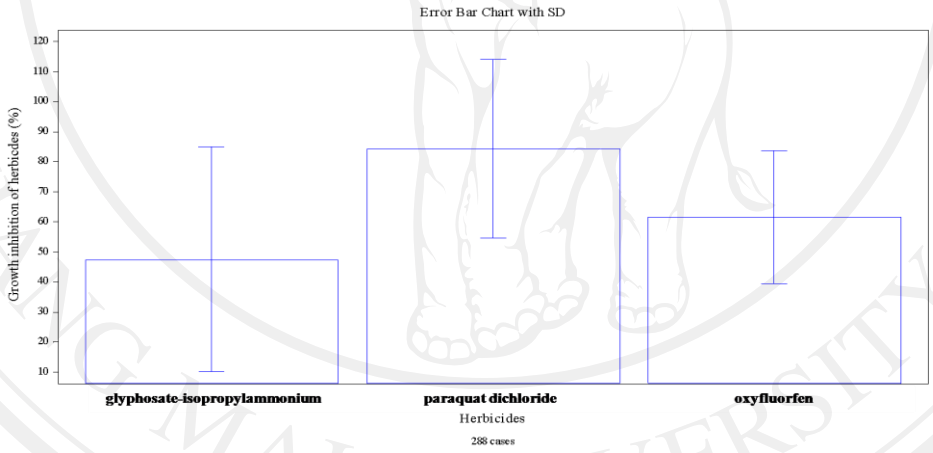

Fig. 3. Effect of various herbicides on growth inhibition of nematophagous fungion Potato Dextrose Agar at 10 days after inoculation. Graph based on the pooled effect of 4 levels (1/3x, 1/2x, 1x, 2x) of the recommended herbicide rate on eight isolates of nematophagous fungi (*P<0.01*).

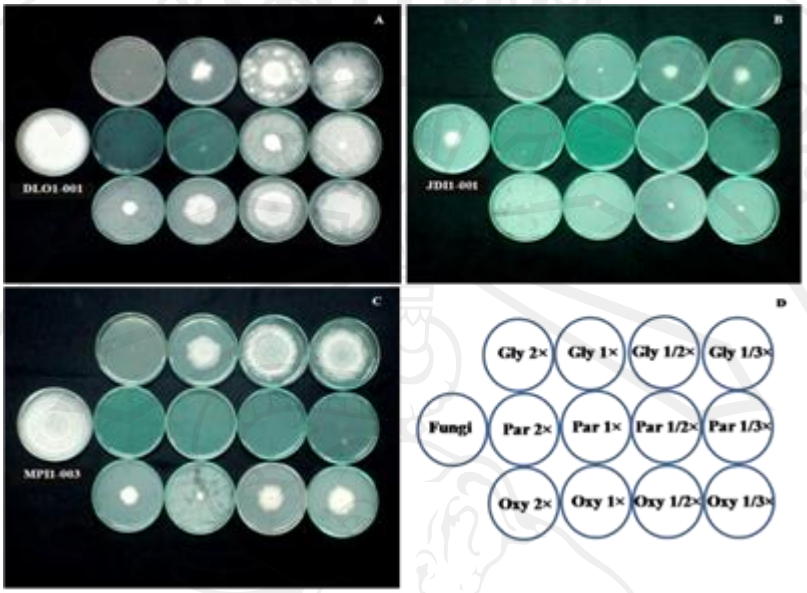

Fig. 4. Sampling of colony characterizations of three nematophagous fungi comparing effect from herbicides on PDA after 10 days incubation. A) *Arthrobotrys oligospora* isolate DLO1-001 B) *Arthrobotrys thaumasium* isolate JDI1-001 C) *Arthrobotrys thaumasium* isolate MPI1-003 and D) Location of tested Petri dish, Gly= glyphosate-isopropylammonium, Par= paraquat dichloride, Oxy= oxyfluorfen, 2x= Double rate, 1x= Recommended rate, 1/2x= Half of recommended rate and 1/3x= One-third of recommended rate.

Discussion

In worldwide agriculture, chemical products may detrimentally affect biocontrol agents. Most pesticides including fungicides, insecticides and herbicides affect growth and development causing abnormalities of many non-target organisms (Wikipedia, 2012i). Goltapeh *et al.* (2008) determined that the fungicides formalin, benomyl, thiophanate methyl and carbendazim and insecticides diflubenzuron and malathion caused 28-100% inhibition of radial mycelial growth of *Arthrobotrys oligospora* in Petri dishes to varying degrees. This research showed that isolates of nematophagous fungi from Thailand also varied with respect to their response to different agrochemicals.

Priority of effect was observed in fungicide phenylamide (metalaxyl mixed with mancozeb) followed by ethyl phosphonate (fosetyl aluminium), but a wide range of pesticide classes including a dithiocarbamate (dazomet), carbamate (carbaryl) organophosphate (chlorpyrifos), aromatic hydrocarbon mixed with thiadiazole (quintozene mixed with etridiazole), bipyridylium (paraquat dichloride) and diphenyl ether (oxyfluorfen) were moderately to highly inhibitory of the growth and sporulation of most fungi tested.

On the other hand, low inhibition by pesticides was often observed in *Paecilomyces* sp. (WJI1-003) and *Pochonia* sp. (KJO1-003) in comparison with the *Arthrobotrys* species examined. This finding is similarly to the result of Jacobs *et al*. (2003), who also observed only weak growth inhibition of these fungi by toclofos methyl. Both fungi rapidly produce numerous small conidia and also have thick, velvety and cottony colonies, respectively, which may act to lessen pesticide inhibition compared to *Arthobotrys* spp. which has a fuzzy or powdery texture. However, no reports indicate that *Paecilomyces* sp. or *Pochonia* sp. release enzymes or metabolites that inactivate toxicants.

Our research results indicate that application of nematophagous fungi as biological agents against root-knot nematodes in plantations using chemicals should be timed with respect to conventional chemical application to avoid inactivation of biological agents concordantly to the conclusion of Kim & Riggs (1998). Furthermore, increasing the amount of bio-agent formulations, by adding a sporulation promoter and/or deploying an inundative approach through frequent bio-control reapplications may help to reduce the effect of agrochemical usage. Other strategies to help ensure the effectiveness of nematophagous fungi as components of an integrated approach to root knot nematode control could also include the use of pesticides which are less inhibitory, reduction of the pesticide rate if feasible and selection of fungal bio-agent isolates that are the least pesticide-sensitive.

Acknowledgements

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